

# APPLICATIONS OF CALCIUM PHOSPHATE NANOPARTICLES IN DRUG DELIVERY SYSTEM

## Abstract

Calcium phosphates (CaP), the major mineral composition of bone and teeth, are exceptionally biocompatible and frequently used in bone tissue engineering. The biodegradable calcium phosphates have a high affinity for nucleic acids and therapeutic prescription drugs. Calcium phosphate nanoparticles (CaPNPs) can also be employed as excellent delivery vehicles to transport therapy into tumor cells due to their high affinity for binding to therapeutic medicines and nucleic acids. CaPs' simplicity of manufacture and functionalization, as well as intrinsic features such as pH dependent solubility, offer benefits in the administration and release of these bioactive compounds employing CaPs as nanocarriers. Because of their safety and biodegradability, CaPNPs are effective carriers for vaccine formulation and adjuvants. A brief account on the usage of CaPNPs as a vehicle of drug delivery for various diseases and also in the field of regenerative medicine has been explained. Many research groups have made significant progress in studying CaPNPs in the context of vaccination. In this study, we also focused on the fundamental properties of CaPNPs as well as their most recent application as a vaccine.

**Keywords:** Calcium phosphate nanoparticle, cancer, drug delivery, immune system, vaccine

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## **I. INTRODUCTION**

Nanoparticles (NPs) are promising drug delivery systems due to their ability to enter and exit biological compartments and reach biological targets. The unique optical, electrical, physicochemical, and biological features of nanoparticles—due to their high surface to volume ratio—have led to their usage as drug delivery systems [1]. Any prospective bioactive substances should be able to be incorporated into a drug carrier through physical or chemical bonding while also being protected in the bloodstream. By adjusting their size, shape, ability to carry cargo, and surface qualities, nanoparticles can be made to carry out a variety of tasks [2]. Potential bioactive substances ought to be able to be incorporated physically or chemically by a drug nanocarrier in order to protect them in the bloodstream. To improve therapeutic effectiveness, the nanocarrier complex should degrade gradually and offer constant and regulated drug release over an extended period of time [3]. Also, it must offer a viable method for binding just to the intended cells or tissues in order to minimize side effects and increase the concentration of the drug at the site [4]. When administered, nanoparticles get into the biological system. The blood, gastrointestinal system, lungs, skin, and muscle are major administration sites. The various areas of the body then receive nanoparticles from the administration site. While some of these nanoparticles approach and reach target cells, others are trapped in off-target locations or are completely eliminated from the body [1]. Numerous biomaterial-based delivery systems or carriers have been produced and are now employed to treat cancer. In nanotechnology, several kinds of nanoparticles, namely polymeric, inorganic, gold etc., and liposomes, hydrogels, and exosomes, are used as drug delivery systems [5].

## **II. CALCIUM PHOSPHATE NANO PARTICLES (CaPNPs)**

Nanoparticles are promising vehicles for the precise delivery of molecular therapies to diseased sites. Nanoparticles interact with a series of tissues and cells before they reach their target, which causes less than 1% of administered nanoparticles to be delivered to these target sites. Among different synthetic nanocarriers, biocompatible calcium phosphate nanoparticles (CaPNPs) have gained interest in nanomedicine which are main mineral composition of human bone and teeth [6].

After cellular absorption, CaPNPs are easily soluble at lower pH inside phagosomes, endosomes, or lysosomes and stable at the neutral pH of our body fluid [7]. CaPNPs have a number of benefits including the capacity to incorporate drug inside and surface of NPs by physical bonding or covalent bonding, the capacity to retain the drug molecules until the NPs come to the target site, and the ability to produce the safe compounds calcium and phosphate ions after CaPNP is degraded [8], [9]. Beginning from the regeneration of damaged bones to the treatment of cancer, calcium phosphate nanoparticles have proven to be an excellent medium of drug delivery.

## **III. CaPNPs FOR INTRACELLULAR DELIVERY FOR TREATMENT OF CANCER**

For the treatment of one of the leading causes of death all across the world, the utilization of calcium phosphate nanoparticles had taken place for delivering drugs to the tumor cells. Cancer is a disease associated with the uncontrolled proliferation of the cells of the human body, leading to the eventual spreading of the same to other parts of the body. The origin of the cancer cell includes almost every tissue inside the human body. Due to the

mutations of either the proto-oncogenes or the tumor suppressor genes, the normal cell cycle, by the dint of which cell division takes place in a healthy condition, becomes uncontrollable and gives rise to cancerous tumor [10], [11]. A projection of 28 million new cases of cancer is being predicted annually by 2040, showing an increment of 54.9% in comparison to 2020 [12]. For 2023, the projections for novel cases of cancer and cancer related deaths are 1,958,310 and 609,820, alone for a developed nation like the United State of America [13]. Even in India the prediction for cases of cancer is 2.08 million in 2040, according to GLOBOCON [14].

- 1. Osteosarcoma:** In an instance of delivering doxorubicin to the 143B osteosarcoma cell line, by Zhou et al., in the year 2016, it was found that using calcium phosphate-phosphorylated adenosine organic-inorganic hybrid microspheres had appreciable therapeutic effects [15]. In 2017, Son et al., performed chemotherapy on MG-63 (human osteosarcoma cell line) using drug-loaded CaP nanoparticles and found that such nanoparticles efficiently released the drugs in a controlled fashion [16]. The usage of calcium phosphate nanoparticles is not limited to the realm of treating osteosarcoma but extends to a myriad of cancers.
- 2. Melanoma:** In 2022, Lima et al., experimented with calcium phosphate nanoparticles which were loaded with doxorubicin and hyaluronic acid to address melanoma. It was reported that melanoma cells (A-375) had damaged DNA with an increase in the area of the nucleus and also showed senescence. Formation of colonies was also deterred on short exposure treatment followed by 14 days of incubation [17]. Xu et al., used hydroxyapatite nanoparticles based chitosan/alginate hydrogel which showed great anti-melanoma effect [18].
- 3. Breast Cancer:** For delivering cisplatin to treat breast cancer, palladium-based hydroxyapatite was formulated by Fathy et al., in 2017. As an outcome of the study, these nanoparticles showed high cytotoxicity against the target cells [19]. For targeted drug delivery to the breast cancer cells, platinum-loaded selenium-doped hydroxyapatite nanoparticles were prepared by Barbanente et al., in 2020. It was a successful attempt at treating breast cancer as this mode of drug delivery was able to selectively prevent the proliferation of cancer cells [20]. Hydroxyapatite-based nanocomposite loaded with quercetin had successfully treated breast cancer, in an experiment conducted by Samadi et al., in 2021. On administering the drug-loaded nanocomposite on MCF-7 cell line, cytotoxicity in the same was observed as quercetin was released in a sustained manner, eventually leading to the apoptosis of the cancer cells [21].
- 4. Cervical Cancer:** For chemotherapy to treat cervical cancer, Zhu et al. experimented with mesoporous silica-based CaPNPs to deliver doxorubicin, to HeLa cell line, after encapsulating with liposome and zinc phthalocyanine. With controlled drug release, cellular uptake of the nanoparticles was reported, which induced apoptosis of the cells due to increased osmotic pressure [22]. On experimenting with HeLa cell line, Rout et al., in 2012, had found that the magnetic calcium phosphate nanoparticles were capable of efficient delivery of cis-platin which induced apoptosis by elevating the levels of toxicity, in the target [23].
- 5. Colorectal Cancer:** In case of treatment of colorectal cancer, CaPNPs have also proven to be efficient vehicles for drug delivery and it can be exemplified by a study conducted

by Mohiyuddin et al., in 2018. On loading 5-fluorouracil on CaPNPs and delivering it to the population of HCT-15 colorectal cancer cells, half of the population of the mentioned cell line were inhibited. Thus, these nanoparticles exhibited anti-neoplastic behavior [24]. The experiment conducted by Bai et al., in 2021, can also be cited as an important one as they had successfully encapsulated PSVII carboxymethyl- $\beta$ -cyclodextrin inclusion compound in CaPNPs to target colon cancer. The drug resistance was inhibited by the increased expression of E-cadherin and a reduction in the N-cadherin and MMP-9 expression was also observed, in this experiment [25]. Another recent example of addressing colon cancer involves the experiment conducted by a recent experiment conducted by Mesas et al. in 2022. T-84 colorectal cancer cell line were subjected to euphorbetin and esculetin (*Euphorbia lathyris* seeds derived coumarin compounds). These coumarin compounds were coated on amorphous calcium phosphate nanoparticles and were tested in both *in vitro* and *in vivo* setup. An elevated anti-tumorigenic activity, characterized by the reduction in the volume of the tumor with poorly developed tumor vasculature and reduced number of polyps was observed, *in vivo* [26]. The help of 3D organoid model has been taken by Deng et al., to provide evidence for the efficacy of drug delivery using hydroxyapatite nanocluster for chemotherapy of colorectal cancer. On loading DOX onto the fabricated hydroxyapatite nanocluster, it was found that the said drug was released in a slightly acidic environment on delivering it to 3D organoid model of colon cancer, showing appreciable antitumor activity [27].

6. **Lung Cancer:** CaPNPs finds its usage even in treating lung cancers also. In the experiment conducted by Mohiyuddin et al., in 2018, 5-fluorouracil loaded CaPNPs were used for delivering the drug to A549 lung adenocarcinoma cells. In this case half of the cell line population was inhibited [24]. Lumefantrine was also delivered to A549 cells, in an experiment by Sethuraman et al., by the usage of CaPNPs loaded on lipidic cubosomes. Here, the cellular uptake of lumefantrine occurred resulting in the eventual apoptosis of the cells due to enhanced cytotoxicity, because of the drug delivery system [28]. In 2020, Li et al., provided evidence of conducting successful chemotherapy of lung cancer by the use of hydroxyapatite nanoparticles loaded with Bovine Serum Albumin as a system of drug delivery [29]. Even for the photodynamic therapy, through the generation of reactive oxygen species (ROS) in the A549 cells of lung cancer, hydroxyapatite nanoparticles, which were doped with hafnium, have proven to be a potential option. The ROS levels in the mentioned cell line were found to have increased on bombardment with ionizing radiation, after they were subjected to the administration of the doped nanoparticles [30].
7. **Prostate Cancer:** For the treatment of prostate cancer also CaPNPs have been developed. Luo et al., in 2010 had successfully activated caspase 2, which had led to the apoptosis of the PC3 prostate cancer cell, by the use of oleic acid coated hydroxyapatite nanoparticles carrying Docetaxel [31]. Yang et al., had developed calcium phosphate based nanoparticle for delivering zoledronate & docetaxel for inhibiting metastasis of prostate cancer to the bone, in an *in vitro* 3D model. The aforesaid nanoparticles were found to have effectively reduced the proliferation of the PC-3 cell line and bone lesion by co-delivering the two drugs, hence proving to be a potential option for treating bone metastasis of prostate cancer [32]. As another potential candidate to target the same disease, nano hydroxyapatite combined with folic acid and polyethylene glycol, was synthesized by Deng et al., recently in 2023. The delivery of the anti-cancer drug Doxorubicin was efficient and apoptotic assay verified its anti-tumor effect. SPECT

showed proper targeting of the prostate cancer cells with bare minimum damaged caused to the healthy tissues [33].

#### **IV. RHEUMATOID ARTHRITIS**

Calcium phosphate nanoparticles have been utilized the most in the treatment of disorders related to the skeletal system and also in its regeneration. One of the most important diseased conditions related to the skeletal system includes rheumatoid arthritis. An incurable, chronic, inflammatory, autoimmune disorder, beginning in the synovial joints and affecting the joints, primarily [34]. The treatment can reduce pain and deter further damage [35]. In the process of developing a treatment, Pandey et al., had fabricated CaPNPs loaded with methotrexate. As an outcome of this process, cartilage and bone regeneration was observed with a significant decrement in the progression of the disease [36]. As another viable option for the treatment of rheumatoid arthritis, using hyaluronic acid coated hydroxyapatite nanoparticles having teriflunomide and methotrexate loaded onto it has emerged recently in 2021. It also has a synergistic effect in the sense that this method of drug delivery also comes with reduced hepatotoxicity [37].

#### **V. REGENERATIVE MEDICINE**

In the field of regenerative medicine, CaPNPs have also proven to be of pivotal importance for the regeneration of bones and blood vessels. In 2018, through an experiment conducted by Chen et al., it was found that CaPNPs loaded with Dexamethasone, induced angiogenesis and osteogenesis in human umbilical vascular endothelial cells placed in scaffolds having microgrooves [38]. Osteoconductive effects were also induced in human adipose derived mesenchymal stromal stem cells, by calcium phosphate nanocrystals, fabricated by Marycz et al.[39]. Contributing to osteogenesis is not the only application of CaPNPs, in the field of regenerative medicine. Salehi et al., in 2017, had successfully developed a hydroxyapatite nanoparticle for regenerating neurons. Type I collagen loaded hydroxyapatite nanoparticles have been reported to have effectively reversed the sciatic nerve crush injury, by regenerating the peripheral nerves [40].

#### **VI. ANTIMICROBIAL ACTIVITY**

CaPNPs have been used to counter microbial toxicity as well. Kanamycin and gentamycin loaded CaP nanoparticles have shown sustained drug release. The results have also revealed that the cell membranes of the bacterial species, involved in the study, have undergone disruption, thus, inhibiting the toxic impact of bacterial infection [41]. CaPNPs have been fabricated as bifunctional system for delivering chlorhexidine to prevent bacterial colonization of dental surface and also to achieve remineralization of the damaged enamel of teeth [42]. Fabricated hybrid hydroxyapatite based nanofibers loaded with Doxycycline has also shown excellent inhibition of both gram negative and gram positive bacteria [43].

- 1. Osteomyelitis:** Osteomyelitis is a musculoskeletal infection which involves the inflammation of bone and bone marrow by pyogenic organisms, which includes bacteria, fungi and mycobacteria, spreading through the fractures, surgery or bloodstream [44], [45]. It can be chronic or acute in nature, characterized by destruction of bone, necrosis & apposition of new bone [44], [45]. It is currently a pressing challenge in the field of orthopedic surgeries [46]. For addressing such infections, cloxacillin was loaded onto a

novel nanocomposite cement based on CaP, synthesised by Seyfoori et al., in 2017. The results of this study showed that there was controlled drug release leading to the inhibition of growth of bacterial population [46]. CaP nanoparticles powder has also been successfully developed for the tunable delivery of bovine serum albumin and fluorescein at the site of infection. It is also being expected with further research osteoconductive properties can also be generated in the mentioned nanoparticles [47]. *S.aureus* being one of the most prominent causative agents of this infection, has been targeted by Uskoković and Desai by loading clindamycin on hydroxyapatite. This endeavor had caused the intracellular population of bacteria to decrease effectively with a slowed down pathogenic growth. Besides this the speed of the process of osteogenesis was also enhanced [48].

- 2. Periodontitis:** Periodontitis is one of the most common inflammatory diseases of the oral cavity, which occurs due to infection of the tissue supporting the tooth structure, called the periodontium [49]–[51]. It is characterized by the formation of biofilms which varies in the composition.[51] For delivering high concentration of antibiotics to treat periodontitis with minimal side effects, hydroxyapatite based nanoparticles were prepared by Madhumathi et al., in 2017. These nanoparticles had shown slow and sustained release of tetracyclin drug and had also shown suitability for effectively managing periodontal infrabony defects [52]. Tetracycline-loaded multifunctional nanocrystalline CaP also exhibited antimicrobial activity against both gram positive and gram negative species of bacteria [39].

## VII. APPLICATION OF CALCIUM PHOSPHATE IN CANCER IMMUNE-VACCINE DEVELOPMENT

Immunization is a long-practiced procedure, this has been functional for centuries. Although vaccines for infectious diseases are in use for many decades, a very limited number of cancer vaccines are approved for human usage. In recent times, some vaccines are seen to cut short in the number of cancer incidences. Cancer immunotherapy has crossed a long pathway since 1909 with Paul Ehrlich proposing the immune surveillance hypothesis to recent most used vaccines in human diseases [53]. Basically, cancer vaccines are designed to induce immune responses towards antigens. Besides very few cancer vaccines being actually available for humans, any of them are in the clinical trial phase. The success rate of cancer immune vaccine is dependent on many factors like types of antigen used, tumour microenvironment, the tumor's immune system, etc [54].

- 1. Calcium Phosphate-Mediated Cancer Immune Vaccine:** In the past twenty years, nanoparticle-mediated vaccine delivery has gained immense popularity. Solid cores which range in diameter from 1 to 1000 nm are used to create nanoparticles. They have showed tremendous potential in functions as vaccine carriers and medication delivery systems. Nanoparticles of calcium phosphate have been used as adjuvants in vaccines for several years. Relyveld and his colleague published the very first study on calcium phosphate vaccination adjuvants in 1964 [55]. Adjuvants for the vaccines against diphtheria, pertussis, poliomyelitis, and tetanus are commonly produced using calcium phosphate nanoparticles [56], [57]. Current research suggests that Cap nanoparticles could potentially replace alum salts in the manufacturing of vaccines [58]. It is simple to create calcium phosphate nanoparticles since it is non-toxic, biodegradable, inexpensive to make, and demonstrates pH-dependent solubility, which is significantly different from alum powders [59]. Also, it is clear that calcium nanoparticles shield antigen cargo from

early proteolytic and enzymatic degradation as well as blocking reticulohistocytic system removal (RHS) [60],[61]. Cap is a good candidate to be a vaccine adjuvant since it is simple to functionalize with different adjuvants [62],[63].

- 2. Antigen Loading Strategies for CaPNPs:** Strong bonds between the antigen and nanoparticle are necessary for nanoparticles to work well as a vaccination adjuvant. For this objective, adsorption, encapsulation, co-mixture, and chemical conjugation are primarily used. The most widely used techniques for antigen loading in nanoparticles are adsorption and encapsulation, which are accomplished by charge attraction and hydrophobic contact [64]. The weak interaction caused by salt concentrations, exogenous lipids, and proteins that displace the antigen at the injection site is a drawback of the adsorption method. This can result in a burst release of antigen from NPs in vivo or a sudden release away from the APC, which lowers immunity [65]. The main factor influencing surface adsorption is the electrostatic interaction between the antigen and CaPNPs. For instance, the electrostatic interaction between the  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  anions of HAP and the  $\text{COO}^-$  and  $\text{NH}_4^+$  cations of the protein antigen causes the adsorption of bovine serum albumin (BSA), ovalbumin (OVA), or lysozyme on HAP [66].

To achieve constant antigen release and delivery of all the antigen to the antigen-presenting cell (APC), encapsulation and conjugation may be a suitable option. Generally speaking, encapsulation may be accomplished by combining antigens with chemical reagents and solvents during the NP synthesis step, producing a strong contact akin to chemical conjugation. As the antigen is not released until the NPs dissolve, this method can increase the effectiveness of antigen transfection by delivering the antigen where it is needed. In a precipitation synthesis, Dasgupta et al. encapsulated BSA protein by first integrating it with the  $\text{Ca}^{2+}$  solution before combining it with the  $\text{PO}_4^{3-}$  aqueous solution. This produced 50–60 nm NPs that contained up to 24% by weight BSA protein [67]. Using a hybrid encapsulating stabilisation technique, Chiu et al. (2012) [68] produced the antigenic protein with peptides that bonded to and stabilised calcium phosphate. When the  $\text{Ca}^{2+}$  phase was added to the  $\text{PO}_4^{3-}$  aqueous solution phase in this manner, the protein stabilised the calcium-phosphate nucleation and created nanoparticles (60–70 nm) with an amorphous calcium phosphate core and a protein shell [69]. Proteins and peptides, which are antigenic molecules, have also been included by chemical conjugation by being covalently linked to the surface of the NP. Ramachandran et al. loaded the PEG-CaP particles with insulin for an oral administration therapy after functionalizing the surface of CaP NPs with carbodiimide chemistry to covalently connect diamino-PEG [70],[71]. To covalently bind antibodies and antigens, Kozlova et al. synthesised CaP NPs covered with a silica shell that was functionalized by thiol/amino groups or salinization [72]. Since the conjugated antigen has been discovered to be protected from proteases in the mucosal tissue, the use of antigen-conjugated NPs has some notable advantages for mucosal immunisation. Contrary to a mixed formulation, conjugation of antigens, adjuvants, and NPs has been found to provide greater immunity. CaP NPs function as an immune potentiator (adjuvant) as well as a delivery method [73],[74].

## VIII. NANOPARTICLE UPTAKE AND IMMUNOGENICITY

The ability to generate an immunological response depends on how well the NPs are taken up by the dendritic cells (DC). Using CaPNPs as vaccine carriers rather than just

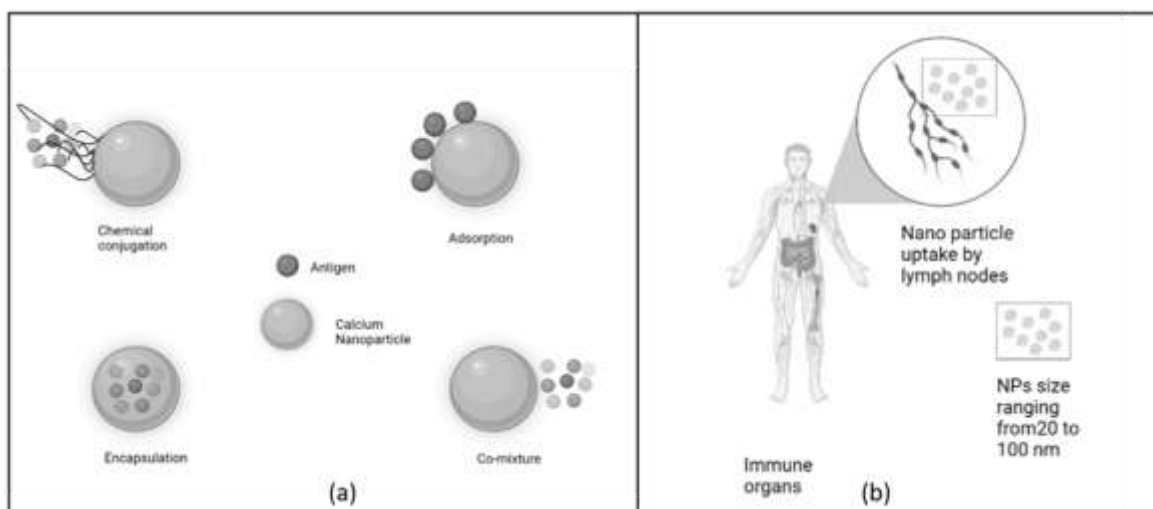
antigens results in a more effective delivery of antigen to DC [75]. The size, shape, composition, and surface charge of the nanoparticles all affect their capacity for nanoparticle absorption. It is clear that the immunogenicity of vaccines is significantly influenced by nanoparticle size [76]. The size of the np is the most significant variable influencing np uptake [77]. NPs are readily absorbed by lymph nodes and the lymphatic system in the size range of 20–100 nm [78]. Once in the lymph nodes, the antigens produced by the nanoparticles can quickly activate B cells and are quickly ingested by DCs by phagocytosis. Long-term humoral and cell-mediated immunity are strengthened by this. Cells (DCs) at the injection site take up larger nanoparticles (size around 200 nm), which are not easily taken up by lymph nodes, by endocytosis or micropinocytosis. This occurs prior to the DCs migrating to the lymph nodes and inducing a significant immunological response [79]. Studies conducted in vitro have shown that NPs with a size of less than 500 nm are suitable for DC absorption [80]. In compared to micron-sized particles, nano-sized particles have also been proven to have greater cell penetration and immune response induction abilities [81]. Recent research has demonstrated that nanoparticles between the sizes of 100 and 400 nm can elicit a stronger immune response than smaller or larger nanoparticles [82]. The surface charge of NP has a significant impact on immune response. Comparatively to neutral or negatively charged nps, positively charged CaPNPs are found to be more readily absorbed by cells [83]. Moreover, the form of the particle is crucial in triggering an immunological response. Obviously, compared to spherical or plate-shaped NPs, knife-shaped CaPNPs are more exciting [84],[82]. While inducing similar humoral immunity to connected antigens in vivo, rod-shaped CaPNPs stimulated more IL-1 $\beta$  production in vitro than spherical NPs [82]. The functionality of the NP is significantly influenced by the configuration of NP. When compared to HAp, amorphous calcium phosphate (ACP) is found to have a weaker immunostimulatory effect [85]. Since CaPNPs are cheaper and their formulation is easily modifiable, they can be widely used as vaccine adjuvants.

## IX. TARGETING INNATE IMMUNE SYSTEM

Innate immune system plays important role in development of adaptive immunity to antigens [86]. In recent past, the effect of CapNPs on innate immunity was studied. In mice model, BSA loaded CaPNPs showed robust stimulation of innate immune response [87]. When mouse was injected with  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) nanoparticles, they initiated migration of mouse monocyte/macrophage cells to injection site, consequently, it largely stimulated the production of macrophage inflammatory protein 1 alpha (MIP-1 $\alpha$ ). This is a suggestive that of  $\beta$ -TCP plays a novel role in vaccine application [88]. Innate immunity greatly depends on pathogen-associated molecular patterns (PAMPs) by Pattern recognition receptors (PRR) like TLRs (Toll like receptors). TLR activation can lead to inflammatory cytokine production, enhance macrophage phagocytosis activity, as well as increase antigen presentation. As a result, TLR agonists in vaccine development is hugely studied in pre clinical stage [89],[90]. The adjuvant effect of CaP encapsulated with TLR ligands CpG and polyriboinosinic:polyribocytidilic acid (poly(I:C)) was studied in mouse model. It was observed that the TLR infused NPs initiated high levels of innate inflammatory cytokines IL-12p70 and TNF- $\alpha$ , this resulted in DC maturation and rapid activation of CD4 $^{+}$  T cells [91]. Flagellin is a PAMP, this can be sensed by TLR 5 on the host surface. Flagellin functionalised CaPNPs initiated more proinflammatory cytokines IL-1 $\beta$  from bone marrow derived macrophages [92],[93]. Over the past ten years, researchers have looked into how CaPNPs can boost the innate immune system. In a study conducted by Behera et al., it was shown that CaPNPs loaded with Labeo rohita H. S-layer protein, derived from *Aeromonas*



hydrophila, induced a strong innate and adaptive immune response in a fish model upon parenteral immunization [94]. According to Matesanz et al., [87] BSA-loaded nanosized CaP caused an immediate enhancement of the innate immune response in mice. Additionally, the production of inflammatory cytokines like IL-6 (interleukin 6) and TNF- $\beta$  (tumor necrosis factor- $\beta$ ) were significantly increased while macrophage proliferation and phagocytic activity were significantly reduced [87]. In the development of calcium phosphate nanoparticle (CaP NP) vaccine formulations, it is important to consider several crucial findings. The inclusion of Toll-like receptor (TLR) ligands in the formulation can not only increase the adjuvanticity of the vaccine but also act as a "depot" system. This depot effect allows the vaccine to stay at the injection site for an extended period, enabling efficient sequestration of the vaccine by dendritic cells and other antigen-presenting cells. The selection of CaP NP synthesis methods is also critical, as various methods can result in variations in the Ca to P ratio, which significantly influences the immunogenicity of the vaccine. It is crucial to note that hydroxyapatite represents one of the most stable CaP formulations. However, considering substitute CaP formulations such as tricalcium phosphate ( $\beta$ -TCP) is also valuable. By incorporating TLR ligands into the CaPNP vaccine formulation, the immune response can be increased due to the activation of innate immune receptors. These ligands can trigger signaling pathways that promote antigen presentation, macrophage phagocytosis activity, and the production of inflammatory cytokines. Consequently, the immune system mounts a stronger and more targeted immune response against the vaccine antigen [95].

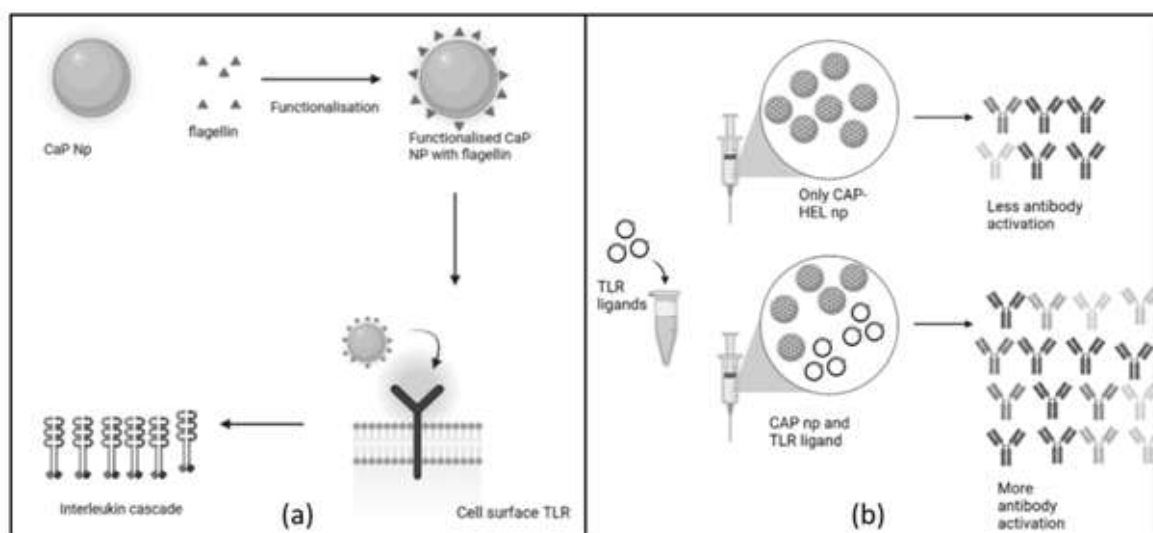


**Figure 1:** (a) Different Strategies for Antigen Loading in CaP nanoparticles (b) NPs are Easily Absorbed by Lymph Nodes and the Lymphatic System in the Size Range of 20–100 nm

## X. HUMORAL IMMUNE SYSTEM ACTIVATION

In comparison to conventional aluminium adjuvants, CaPNPs initiate more antibody response [96]. Avian avulavirus-1 conjugated CaPNPs vaccines were applied to chickens in an experiment by Viswanathan et al and that induced greater and quicker humoral immune response (IgA antibody) [97]. In a different experiment, foot and mouth disease virus targeting DNA vaccine was encapsulated in CaP NP and it was injected in mouse and guineapigs, as a result a powerful immune response (neutralizing antibody) was initiated and it protected the animals from live viruses [98]. In a study by Temchura et al, Hen Egg

Lysozyme (HEL) conjugated CaP NPs efficiently targeted and activated B cells both in vitro and in vivo by using BCR-transgenic B cells from SW-HEL mice model. The expression of B-cell activation markers were significantly higher as a result of the application.[99] In an experiment done by Chua et al, a novel vaccine was developed using OVA as a model antigen as well as chitosan and HAP NPs as delivery medium. Once the vaccine was introduced in mouse, a powerful antibody response (IgG1) was induced and this it remained for 12 months [100]. In another studies, it was seen that CaP-HEL (calcium phosphate-hen egg lysozyme) NPs encapsulated with TLR ligands initiated higher level of antibody response (IgG) in comparison to when Cap-HEL NPs were not functionalised with TLR ligand. The authors of this study also evaluated several TLR ligands for CaP-HEL and found that TLR ligands loaded with CaP-HEL resulted in greater IgG antibody levels than CaP-HEL used alone did. The study showed that CaP-HEL NPs functionalized with various TLR ligands influenced the generation of mucosal IgA antibodies as well as the IgG isotype response.



**Figure 2:** (a) CaPNP Functionalised with Flagellin after Binding with TLR creates Interleukin Cascade. (b) More Antibody Activation when CaP-HEL nps Functionalised with TLR Ligands

Three Brucella antigens (FliC, 7-HSDH, and BhuA) and two multi-epitopes (poly B and poly T) were loaded into a unique CaP vaccine, and it was shown that this dramatically improved both the cellular and humoral immune responses to the antigens [101]. Additionally, the proportion of IgG2a antigen-specific to IgG1 increased [102]. A new multiepitope (MIC3, ROP8, and SAG1) vaccination with CaP adjuvants was recently developed by Dodangeh et al. They discovered that mice given the MRS-CaP vaccination had higher humoral (IgG1 and IgG2a) and T helper type 1 cell-mediated immunological responses compared to control mice [103]. Overall, the results demonstrate that antigen- and TLR-loaded CaPNPs are potential vaccine formulations for effective antibody production and regulation.

## XI. FUTURE SCOPE AND CONCLUDING REMARKS

Based on the possibility of loading various types of drugs on CaPNPs, it can be predicted that such nanoparticles can serve as an effective medium of drug delivery, for

treating variety of diseases [104], [105]. Bio-imaging using CapNPs also seems to be possible as a part of their clinical application, in the near future [105]. One of the prime applications in the realm of regenerative medicine can also include the regeneration of bones, mainly because of their property of being biocompatible and of supplying calcium ions, naturally [104]. Despite great strides in the development of these nanoparticles, the obstacle of stability of the nanostructure along with a steady circulation in the bloodstream, which is naturally rife with proteins, enzymes and cells of the immune system, still remains [106]. Agglomeration of the nanoparticles due to high ionic strength and low electrostatic repulsion between the particles is another hurdle that requires attention.[106] Another drawback is the alterations of the surface characteristics as a result of the protein adsorption, in this case, which needs to be addressed [106]. In spite of the presence of such hurdles, it is necessary that there is a development on the understanding of the fundamental concepts of chemistry, biology and properly designed animal studies, supported by appropriate stewardship to enable the delivery of drugs using CaPNPs to their target site in a precise manner [106].

Cancer vaccines must be secure, efficient, and economical, and calcium nanoparticles considerably takes part in these goals thanks to their security, controlled release, targeting of DCs (dendritic cells), better antigen absorption, and increased immunogenicity. Here, we went through the factors to take into account while developing calcium nanoparticles as cancer vaccine delivery vehicles. The modulation of antigen distribution, cellular uptake mechanism, antigen presentation, and the type and strength of immune response have all been shown to depend critically on the physicochemical properties of size, zeta potential, particle rigidity, targeting ligand, and intrinsic immunogenicity. Therefore, it is important to consider these traits in order to maximize antitumor responses. We also included recent developments in nanotechnology for the administration of cancer vaccines, and we hope that this study will provide direction for the development of future cancer vaccines. However, the balance between the host immune system's anti-tumor response and the suppressive immunological microenvironment complicates tumor growth [107]. Therefore, combining cancer vaccines with therapies that limit the cancer microenvironment would further improve the immunotherapeutic result, and this combination has received substantial preclinical and clinical evaluation [108]. Combining cancer vaccines with checkpoint inhibitor blockades is one of these strategies. It is widely established that ligation of PD-1 with PD-L1 results in T cell fatigue, tolerance, and malfunction [109]. Blocking these inhibitory pathways should boost the T cell-mediated immune response, which is thought to be the primary cancer vaccine effector. Another potential for pairing with cancer vaccination would be approaches to enhance effective T cells infiltration while preventing the actions of suppressive immune cells inside the tumour microenvironment. The majority of treatment failures for cancer vaccines, checkpoint blockades, and CART therapies have been linked to the inadequate infiltration of CD8+ T lymphocytes in over 70% of malignancies (particularly solid tumors), according to a number of literature studies and clinical trials [110]. Tyrosine kinase inhibitors against vascular endothelial growth factor, modest kinase inhibitors against STAT3, and antibodies or RNA interference that modify the suppressive immunological milieu have all been shown to dramatically increase tumor regression and survival [111]. Chemotherapeutic drugs are a further feasible alternative for combination therapy. Chemotherapeutic drugs with immunomodulatory characteristics, such cisplatin, docetaxel, and doxorubicin, can boost vaccine-mediated anticancer immune responses. The types of medications and the particular vaccines used, together with the dose regimen for each modality, all affect how synergy works. Combination treatment may benefit from the flexibility of nanocarriers to the fullest extent possible. It is important to note that the choice of more potent cancer vaccines and the

time of the combination are crucial factors that might significantly affect the clinical antitumor effect [112]–[114].

Therefore, future work might concentrate on improving calcium phosphate nanoparticles for therapeutic applications including assessing systemic toxicity and conducting pharmacokinetic and pharmacodynamics research. Further attempts will begin path for CaPNP clinical applications for drug delivery applications in the near future. The use of CaPNPs will provide a promising new platform and delivery system for the manufacturing of efficient vaccinations against cancer and infectious diseases.

## REFERENCE

- [1] J. L. Y. Wu, B. P. Stordy, L. N. M. Nguyen, C. P. Deutschman, and W. C. W. Chan, “A proposed mathematical description of in vivo nanoparticle delivery,” *Adv. Drug Deliv. Rev.*, vol. 189, p. 114520, Oct. 2022, doi: 10.1016/j.addr.2022.114520.
- [2] A. Albanese, P. S. Tang, and W. C. W. Chan, “The Effect of Nanoparticle Size, Shape, and Surface Chemistry on Biological Systems,” *Annu. Rev. Biomed. Eng.*, vol. 14, no. 1, pp. 1–16, 2012, doi: 10.1146/annurev-bioeng-071811-150124.
- [3] C. Qiu *et al.*, “Preparation and application of calcium phosphate nanocarriers in drug delivery,” *Mater. Today Bio*, vol. 17, p. 100501, Dec. 2022, doi: 10.1016/j.mtbio.2022.100501.
- [4] J. Yang, X. Wang, B. Wang, K. Park, K. Wooley, and S. Zhang, “Challenging the fundamental conjectures in nanoparticle drug delivery for chemotherapy treatment of solid cancers,” *Adv. Drug Deliv. Rev.*, vol. 190, p. 114525, Nov. 2022, doi: 10.1016/j.addr.2022.114525.
- [5] K. Vyas, M. Rathod, and M. M. Patel, “Insight on nano drug delivery systems with targeted therapy in treatment of oral cancer,” *Nanomedicine Nanotechnol. Biol. Med.*, vol. 49, p. 102662, Apr. 2023, doi: 10.1016/j.nano.2023.102662.
- [6] N. Eliaz and N. Metoki, “Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Applications,” *Mater. Basel Switz.*, vol. 10, no. 4, p. 334, Mar. 2017, doi: 10.3390/ma10040334.
- [7] L.-J. Yi, J.-F. Li, M.-G. Ma, and Y.-J. Zhu, “Nanostructured Calcium-based Biomaterials and their Application in Drug Delivery,” *Curr. Med. Chem.*, vol. 27, no. 31, pp. 5189–5212, 2020, doi: 10.2174/0929867326666190222193357.
- [8] D. Huang, B. He, and P. Mi, “Calcium phosphate nanocarriers for drug delivery to tumors: imaging, therapy and theranostics,” *Biomater. Sci.*, vol. 7, no. 10, pp. 3942–3960, Oct. 2019, doi: 10.1039/c9bm00831d.
- [9] D. C. Bassett, T. E. Robinson, R. J. Hill, L. M. Grover, and J. E. Barralet, “Self-assembled calcium pyrophosphate nanostructures for targeted molecular delivery,” *Biomater. Adv.*, vol. 140, p. 213086, Sep. 2022, doi: 10.1016/j.bioadv.2022.213086.
- [10] “What Is Cancer? - NCI,” Sep. 17, 2007. <https://www.cancer.gov/about-cancer/understanding/what-is-cancer> (accessed Aug. 08, 2023).
- [11] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter, “The Molecular Basis of Cancer-Cell Behavior,” in *Molecular Biology of the Cell. 4th edition*, Garland Science, 2002. Accessed: Aug. 08, 2023. [Online]. Available: <https://www.ncbi.nlm.nih.gov/books/NBK26902/>
- [12] “Worldwide cancer incidence statistics,” *Cancer Research UK*, May 14, 2015. <https://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer/incidence> (accessed Aug. 08, 2023).
- [13] R. L. Siegel, K. D. Miller, N. S. Wagle, and A. Jemal, “Cancer statistics, 2023,” *CA. Cancer J. Clin.*, vol. 73, no. 1, pp. 17–48, Jan. 2023, doi: 10.3322/caac.21763.
- [14] K. Sathishkumar, M. Chaturvedi, P. Das, S. Stephen, and P. Mathur, “Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India,” *Indian J. Med. Res.*, vol. 156, no. 4 & 5, pp. 598–607, 2022, doi: 10.4103/ijmr.ijmr\_1821\_22.
- [15] Z.-F. Zhou *et al.*, “Calcium phosphate-phosphorylated adenosine hybrid microspheres for anti-osteosarcoma drug delivery and osteogenic differentiation,” *Biomaterials*, vol. 121, pp. 1–14, Mar. 2017, doi: 10.1016/j.biomaterials.2016.12.031.
- [16] K. D. Son and Y.-J. Kim, “Anticancer activity of drug-loaded calcium phosphate nanocomposites against human osteosarcoma,” *Biomater. Res.*, vol. 21, p. 13, 2017, doi: 10.1186/s40824-017-0099-1.

- [17] I. B. Lima, B. M. Alvarenga, P. I. S. de Tótaró, F. Boratto, E. A. Leite, and P. P. G. Guimaraes, "Improved antiproliferative activity of doxorubicin-loaded calcium phosphate nanoparticles against melanoma cells." *bioRxiv*, p. 2022.04.21.489118, Apr. 22, 2022. doi: 10.1101/2022.04.21.489118.
- [18] K. Xu *et al.*, "Anti-melanoma effect and action mechanism of a novel chitosan-based composite hydrogel containing hydroxyapatite nanoparticles," *Regen. Biomater.*, vol. 9, p. rbac050, Jan. 2022, doi: 10.1093/rb/rbac050.
- [19] A. A. Fathy, I. S. Butler, M. Abd Elrahman, B. J. Jean-Claude, and S. I. Mostafa, "Anticancer evaluation and drug delivery of new palladium(II) complexes based on the chelate of alendronate onto hydroxyapatite nanoparticles," *Inorganica Chim. Acta*, vol. 473, pp. 44–50, Mar. 2018, doi: 10.1016/j.ica.2017.12.015.
- [20] A. Barbanente *et al.*, "Platinum-loaded, selenium-doped hydroxyapatite nanoparticles selectively reduce proliferation of prostate and breast cancer cells co-cultured in the presence of stem cells," *J. Mater. Chem. B*, vol. 8, no. 14, pp. 2792–2804, Apr. 2020, doi: 10.1039/D0TB00390E.
- [21] A. Samadi, M. Pourmadadi, F. Yazdian, H. Rashedi, M. Navaei-Nigjeh, and T. Eufrazio-da-silva, "Ameliorating quercetin constraints in cancer therapy with pH-responsive agarose-polyvinylpyrrolidone - hydroxyapatite nanocomposite encapsulated in double nanoemulsion," *Int. J. Biol. Macromol.*, vol. 182, pp. 11–25, Jul. 2021, doi: 10.1016/j.ijbiomac.2021.03.146.
- [22] J. Ma *et al.*, "Novel Core-Interlayer-Shell DOX/ZnPc Co-loaded MSNs@ pH-Sensitive CaP@PEGylated Liposome for Enhanced Synergetic Chemo-Photodynamic Therapy," *Pharm. Res.*, vol. 35, no. 3, p. 57, Feb. 2018, doi: 10.1007/s11095-017-2295-z.
- [23] S. R. Rout, B. Behera, T. K. Maiti, and S. Mohapatra, "Multifunctional magnetic calcium phosphate nanoparticles for targeted platin delivery," *Dalton Trans.*, vol. 41, no. 35, pp. 10777–10783, Aug. 2012, doi: 10.1039/C2DT30984J.
- [24] S. Mohiyuddin, S. Naqvi, and G. Packirisamy, "Enhanced antineoplastic/therapeutic efficacy using 5-fluorouracil-loaded calcium phosphate nanoparticles," *Beilstein J. Nanotechnol.*, vol. 9, pp. 2499–2515, Sep. 2018, doi: 10.3762/bjnano.9.233.
- [25] S. Bai *et al.*, "MCP mediated active targeting calcium phosphate hybrid nanoparticles for the treatment of orthotopic drug-resistant colon cancer," *J. Nanobiotechnology*, vol. 19, no. 1, p. 367, Nov. 2021, doi: 10.1186/s12951-021-01115-9.
- [26] C. Mesas *et al.*, "Colon cancer therapy with calcium phosphate nanoparticles loading bioactive compounds from *Euphorbia lathyris*: In vitro and in vivo assay," *Biomed. Pharmacother. Biomedicine Pharmacother.*, vol. 155, p. 113723, Nov. 2022, doi: 10.1016/j.biopha.2022.113723.
- [27] T. Deng *et al.*, "DOX-loaded hydroxyapatite nanoclusters for colorectal cancer (CRC) chemotherapy: Evaluation based on the cancer cells and organoids," *SLAS Technol.*, vol. 28, no. 1, pp. 22–31, Feb. 2023, doi: 10.1016/j.slant.2022.10.002.
- [28] V. Sethuraman, K. Janakiraman, V. Krishnaswami, S. Natesan, and R. Kandasamy, "pH responsive delivery of lumefantrine with calcium phosphate nanoparticles loaded lipidic cubosomes for the site specific treatment of lung cancer," *Chem. Phys. Lipids*, vol. 224, p. 104763, Nov. 2019, doi: 10.1016/j.chemphyslip.2019.03.016.
- [29] G. Li, D. Tang, D. Wang, C. Xu, and D. Liu, "Effective Chemotherapy of Lung Cancer Using Bovine Serum Albumin-Coated Hydroxyapatite Nanoparticles," *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.*, vol. 27, pp. e919716-1-e919716-8, May 2020, doi: 10.12659/MSM.919716.
- [30] M.-H. Chen *et al.*, "Hafnium-doped hydroxyapatite nanoparticles with ionizing radiation for lung cancer treatment," *Acta Biomater.*, vol. 37, pp. 165–173, Jun. 2016, doi: 10.1016/j.actbio.2016.04.004.
- [31] Y. Luo *et al.*, "Docetaxel loaded oleic acid-coated hydroxyapatite nanoparticles enhance the docetaxel-induced apoptosis through activation of caspase-2 in androgen independent prostate cancer cells," *J. Controlled Release*, vol. 147, no. 2, pp. 278–288, Oct. 2010, doi: 10.1016/j.jconrel.2010.07.108.
- [32] Q. Yang *et al.*, "Bone-Targeted Calcium Phosphate-Polymer Hybrid Nanoparticle Co-Deliver Zoledronate and Docetaxel to Treat Bone Metastasis of Prostate Cancer," *J. Pharm. Sci.*, vol. 110, no. 2, pp. 876–887, Feb. 2021, doi: 10.1016/j.xphs.2020.11.005.
- [33] H. Deng *et al.*, "In vitro and in vivo Evaluation of Folic Acid Modified DOX-Loaded 32P-nHA Nanoparticles in Prostate Cancer Therapy," *Int. J. Nanomedicine*, vol. 18, pp. 2003–2015, Dec. 2023, doi: 10.2147/IJN.S403887.
- [34] K. Chauhan, J. S. Jandu, L. H. Brent, and M. A. Al-Dhahir, "Rheumatoid Arthritis," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. Accessed: Jun. 25, 2023. [Online]. Available: <http://www.ncbi.nlm.nih.gov/books/NBK441999/>
- [35] J. Bullock *et al.*, "Rheumatoid Arthritis: A Brief Overview of the Treatment," *Med. Princ. Pract.*, vol. 27, no. 6, pp. 501–507, Mar. 2019, doi: 10.1159/000493390.

- [36] [36] S. Pandey, A. Mahtab, V. Kumar, F. Jalees Ahmad, A. Kamra Verma, and S. Talegaonkar, "Design and development of bioinspired calcium phosphate nanoparticles of MTX: pharmacodynamic and pharmacokinetic evaluation," *Drug Dev. Ind. Pharm.*, vol. 45, no. 7, pp. 1181–1192, Jul. 2019, doi: 10.1080/03639045.2019.1602139.
- [37] S. Pandey *et al.*, "Hyaluronate-functionalized hydroxyapatite nanoparticles laden with methotrexate and teriflunomide for the treatment of rheumatoid arthritis," *Int. J. Biol. Macromol.*, vol. 171, pp. 502–513, Feb. 2021, doi: 10.1016/j.ijbiomac.2020.12.204.
- [38] Y. Chen, S. Chen, N. Kawazoe, and G. Chen, "Promoted Angiogenesis and Osteogenesis by Dexamethasone-loaded Calcium Phosphate Nanoparticles/Collagen Composite Scaffolds with Microgroove Networks," *Sci. Rep.*, vol. 8, no. 1, Art. no. 1, Sep. 2018, doi: 10.1038/s41598-018-32495-y.
- [39] K. Marycz *et al.*, "Multifunctional nanocrystalline calcium phosphates loaded with Tetracycline antibiotic combined with human adipose derived mesenchymal stromal stem cells (hASCs)," *Mater. Sci. Eng. C*, vol. 69, pp. 17–26, Dec. 2016, doi: 10.1016/j.msec.2016.06.051.
- [40] M. Salehi *et al.*, "Regeneration of sciatic nerve crush injury by a hydroxyapatite nanoparticle-containing collagen type I hydrogel," *J. Physiol. Sci.*, vol. 68, no. 5, Art. no. 5, Sep. 2018, doi: 10.1007/s12576-017-0564-6.
- [41] S. Sathya, Y. T. Lim, S. Parthasarathi, P. M. Sivakumar, and R.-S. Kaarmukhil Nilavan, "Fabrication of Drug-Loaded Calcium Phosphate Nanoparticles: An Investigation of Microbial Toxicity," *J. Clust. Sci.*, vol. 33, no. 5, pp. 2009–2018, Sep. 2022, doi: 10.1007/s10876-021-02104-6.
- [42] A. Kovtun *et al.*, "Chlorhexidine-loaded calcium phosphate nanoparticles for dental maintenance treatment: combination of mineralising and antibacterial effects," *RSC Adv.*, vol. 2, no. 3, pp. 870–875, Jan. 2012, doi: 10.1039/C1RA00955A.
- [43] R. Ramírez-Agudelo *et al.*, "Hybrid nanofibers based on poly-caprolactone/gelatin/hydroxyapatite nanoparticles-loaded Doxycycline: Effective anti-tumoral and antibacterial activity," *Mater. Sci. Eng. C*, vol. 83, pp. 25–34, Feb. 2018, doi: 10.1016/j.msec.2017.08.012.
- [44] I. I. Momodu and V. Savaliya, "Osteomyelitis," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. Accessed: Jun. 25, 2023. [Online]. Available: <http://www.ncbi.nlm.nih.gov/books/NBK532250/>
- [45] M. C. Birt, D. W. Anderson, E. B. Toby, and J. Wang, "Osteomyelitis: Recent advances in pathophysiology and therapeutic strategies," *J. Orthop.*, vol. 14, no. 1, pp. 45–52, Oct. 2016, doi: 10.1016/j.jor.2016.10.004.
- [46] A. Seyfoori, A. A. Imani Fooladi, and H. Mahmoodzadeh Hosseini, "Calcium phosphate-based nanocomposite carriers for local antibiotic delivery against an osteomyelitis agent," *Adv. Appl. Ceram.*, vol. 116, no. 6, pp. 316–324, Aug. 2017, doi: 10.1080/17436753.2017.1317508.
- [47] V. Uskoković and T. A. Desai, "Phase composition control of calcium phosphate nanoparticles for tunable drug delivery kinetics and treatment of osteomyelitis. I. Preparation and drug release," *J. Biomed. Mater. Res. A*, vol. 101, no. 5, pp. 1416–1426, May 2013, doi: 10.1002/jbm.a.34426.
- [48] V. Uskoković and T. A. Desai, "Simultaneous bactericidal and osteogenic effect of nanoparticulate calcium phosphate powders loaded with clindamycin on osteoblasts infected with *Staphylococcus aureus*," *Mater. Sci. Eng. C*, vol. 37, pp. 210–222, Apr. 2014, doi: 10.1016/j.msec.2014.01.008.
- [49] N. Mehrotra and S. Singh, "Periodontitis," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. Accessed: Jun. 26, 2023. [Online]. Available: <http://www.ncbi.nlm.nih.gov/books/NBK541126/>
- [50] N. S. Gasner and R. S. Schure, "Periodontal Disease," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. Accessed: Jun. 26, 2023. [Online]. Available: <http://www.ncbi.nlm.nih.gov/books/NBK554590/>
- [51] E. Könönen, M. Gursoy, and U. K. Gursoy, "Periodontitis: A Multifaceted Disease of Tooth-Supporting Tissues," *J. Clin. Med.*, vol. 8, no. 8, p. 1135, Jul. 2019, doi: 10.3390/jcm8081135.
- [52] K. Madhumathi, L. Jeevana Rekha, and T. S. Sampath Kumar, "Tailoring antibiotic release for the treatment of periodontal infrabony defects using bioactive gelatin-alginate/apatite nanocomposite films," *J. Drug Deliv. Sci. Technol.*, vol. 43, pp. 57–64, Feb. 2018, doi: 10.1016/j.jddst.2017.09.015.
- [53] P. Valent *et al.*, "Paul Ehrlich (1854-1915) and His Contributions to the Foundation and Birth of Translational Medicine," *J. Innate Immun.*, vol. 8, no. 2, pp. 111–120, 2016, doi: 10.1159/000443526.
- [54] E. Grimmett *et al.*, "Cancer vaccines: past, present and future; a review article," *Discov. Oncol.*, vol. 13, no. 1, p. 31, May 2022, doi: 10.1007/s12672-022-00491-4.
- [55] E. H. Relyveld, E. Hénocq, and M. Raynaud, "Etude de la vaccination antidiphthérique de sujets allergiques, avec une anatoxine pure adsorbée sur phosphate de calcium," *Bull. World Health Organ.*, vol. 30, no. 3, pp. 321–325, 1964.

- [56] P. Coursaget, B. Yvonnet, E. H. Relyveld, J. L. Barres, I. Diop-Mar, and J. P. Chiron, "Simultaneous administration of diphtheria-tetanus-pertussis-polio and hepatitis B vaccines in a simplified immunization program: immune response to diphtheria toxoid, tetanus toxoid, pertussis, and hepatitis B surface antigen," *Infect. Immun.*, vol. 51, no. 3, pp. 784–787, Mar. 1986, doi: 10.1128/iai.51.3.784-787.1986.
- [57] R. K. Gupta and G. R. Siber, "Adjuvants for human vaccines—current status, problems and future prospects," *Vaccine*, vol. 13, no. 14, pp. 1263–1276, Jan. 1995, doi: 10.1016/0264-410X(95)00011-O.
- [58] A. M. Issa, M. S. Salim, H. Zidan, A. F. Mohamed, and A.-R. H. Farrag, "Evaluation of the Effects of Aluminum Phosphate and Calcium Phosphate Nanoparticles as Adjuvants in Vaccinated Mice," *Int. J. Chem. Eng. Appl.*, vol. 5, no. 5, pp. 367–373, 2014, doi: 10.7763/ijcea.2014.v5.411.
- [59] Y. Lin, X. Wang, X. Huang, J. Zhang, N. Xia, and Q. Zhao, "Calcium phosphate nanoparticles as a new generation vaccine adjuvant," *Expert Rev. Vaccines*, vol. 16, no. 9, pp. 895–906, 2017, doi: 10.1080/14760584.2017.1355733.
- [60] A. K. Salem, "Nanoparticles in Vaccine Delivery," *AAPS J.*, vol. 17, no. 2, pp. 289–291, 2015, doi: 10.1208/s12248-015-9720-1.
- [61] I. Posadas, S. Monteagudo, and V. Ceña, "Nanoparticles for brain-specific drug and genetic material delivery, imaging and diagnosis," *Nanomed.*, vol. 11, no. 7, pp. 833–849, Apr. 2016, doi: 10.2217/nmm.16.15.
- [62] Z. Xu, S. Ramishetti, Y. C. Tseng, S. Guo, Y. Wang, and L. Huang, "Multifunctional nanoparticles co-delivering Trp2 peptide and CpG adjuvant induce potent cytotoxic T-lymphocyte response against melanoma and its lung metastasis," *J. Controlled Release*, vol. 172, no. 1, pp. 259–265, 2013, doi: 10.1016/j.jconrel.2013.08.021.
- [63] Z. Xu, Y. Wang, L. Zhang, and L. Huang, "Nanoparticle-delivered transforming growth factor- $\beta$  siRNA enhances vaccination against advanced melanoma by modifying tumor microenvironment," *ACS Nano*, vol. 8, no. 4, pp. 3636–3645, 2014, doi: 10.1021/nn500216y.
- [64] L. Zhao *et al.*, "Nanoparticle vaccines," *Vaccine*, vol. 32, no. 3, pp. 327–337, 2014, doi: 10.1016/j.vaccine.2013.11.069.
- [65] N. Kamaly, B. Yameen, J. Wu, and O. C. Farokhzad, "Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release," 2015, doi: 10.1021/acs.chemrev.5b00346.
- [66] Y. Boonsongrit *et al.*, "Controlled release of bovine serum albumin from hydroxyapatite microspheres for protein delivery system," vol. 148, pp. 162–165, 2008, doi: 10.1016/j.mseb.2007.09.006.
- [67] S. Dasgupta, S. S. Banerjee, A. Bandyopadhyay, and S. Bose, "Zn- and Mg-Doped Hydroxyapatite Nanoparticles for Controlled Release of Protein," vol. 26, no. 11, pp. 4958–4964, 2010, doi: 10.1021/la903617e.
- [68] D. Chiu *et al.*, "Biom mineralization and size control of stable calcium phosphate core-protein shell nanoparticles: potential for vaccine applications," *Bioconjug. Chem.*, vol. 23, no. 3, pp. 610–617, Mar. 2012, doi: 10.1021/bc200654v.
- [69] J. Li, Y. Yang, and L. Huang, "Calcium Phosphate Nanoparticles with an Asymmetric Lipid Bilayer Coating for siRNA Delivery to the Tumor," *J. Controlled Release*, vol. 158, no. 1, pp. 108–114, Feb. 2012, doi: 10.1016/j.jconrel.2011.10.020.
- [70] B. Slütter, P. Christiaan, Z. Ding, R. Verheul, W. Hennink, and W. Jiskoot, "Conjugation of ovalbumin to trimethyl chitosan improves immunogenicity of the antigen," *J. Controlled Release*, vol. 143, no. 2, pp. 207–214, 2010, doi: 10.1016/j.jconrel.2010.01.007.
- [71] R. Ramachandran, W. Paul, and C. P. Sharma, "Synthesis and Characterization of PEGylated Calcium Phosphate Nanoparticles for Oral Insulin Delivery," pp. 41–48, 2008, doi: 10.1002/jbm.b.31241.
- [72] J. M. Chem and M. Epple, "Journal of Materials Chemistry," pp. 396–404, 2012, doi: 10.1039/c1jm14683a.
- [73] G. Navarro-tovar, G. Palestino, S. Rosales-mendoza, G. Palestino, and S. Rosales-mendoza, "An overview on the role of silica-based materials in vaccine development An overview on the role of silica-based materials in vaccine development," vol. 0584, no. May, 2016, doi: 10.1080/14760584.2016.1188009.
- [74] C. Nembrini, A. Stano, K. Y. Dane, M. Ballester, A. J. Van Der Vlies, and B. J. Marsland, "Nanoparticle conjugation of antigen enhances cytotoxic T-cell responses in pulmonary vaccination," 2011, doi: 10.1073/pnas.1104264108.
- [75] S. V. Dorozhkin, "Calcium phosphates and human beings," *J. Chem. Educ.*, vol. 83, no. 5, pp. 713–719, 2006, doi: 10.1021/ed083p713.

- [76] C. He, Y. Hu, L. Yin, C. Tang, and C. Yin, "Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles," *Biomaterials*, vol. 31, no. 13, pp. 3657–3666, 2010, doi: 10.1016/j.biomaterials.2010.01.065.
- [77] L. Powles *et al.*, "Pullulan-coated iron oxide nanoparticles for blood-stage malaria vaccine delivery," *Vaccines*, vol. 8, no. 4, pp. 1–15, 2020, doi: 10.3390/vaccines8040651.
- [78] T. Knuschke *et al.*, "Prophylactic and therapeutic vaccination with a nanoparticle-based peptide vaccine induces efficient protective immunity during acute and chronic retroviral infection," *Nanomedicine Nanotechnol. Biol. Med.*, vol. 10, no. 8, pp. 1787–1798, 2014, doi: 10.1016/j.nano.2014.06.014.
- [79] T. Knuschke, M. Epple, and A. M. Westendorf, "The type of adjuvant strongly influences the T-cell response during nanoparticle-based immunization," *Hum. Vaccines Immunother.*, vol. 10, no. 1, pp. 164–169, 2014, doi: 10.4161/hv.26203.
- [80] C. Foged, B. Brodin, S. Frokjaer, and A. Sundblad, "Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model," *Int. J. Pharm.*, vol. 298, no. 2, pp. 315–322, 2005, doi: 10.1016/j.ijpharm.2005.03.035.
- [81] S. Sharma *et al.*, "An insight into functionalized calcium based inorganic nanomaterials in biomedicine: Trends and transitions," *Colloids Surf. B Biointerfaces*, vol. 133, pp. 120–139, 2015, doi: 10.1016/j.colsurfb.2015.05.014.
- [82] M. Hayashi *et al.*, "Optimization of physiological properties of hydroxyapatite as a vaccine adjuvant," *Vaccine*, vol. 34, no. 3, pp. 306–312, 2016, doi: 10.1016/j.vaccine.2015.11.059.
- [83] L. Chen, J. M. Mccrate, J. C. M. Lee, and H. Li, "The role of surface charge on the uptake and biocompatibility of hydroxyapatite nanoparticles with osteoblast cells," *Nanotechnology*, vol. 22, no. 10, 2011, doi: 10.1088/0957-4484/22/10/105708.
- [84] M. Ramesh, L. F. Turner, R. Yadav, T. V. Rajan, A. T. Vella, and L. T. Kuhn, "Effects of the physico-chemical nature of two biomimetic crystals on the innate immune response," *Int. Immunopharmacol.*, vol. 7, no. 13, pp. 1617–1629, 2007, doi: 10.1016/j.intimp.2007.08.011.
- [85] Q. Hu *et al.*, "Effect of crystallinity of calcium phosphate nanoparticles on adhesion, proliferation, and differentiation of bone marrow mesenchymal stem cells," *J. Mater. Chem.*, vol. 17, no. 44, pp. 4690–4698, 2007, doi: 10.1039/b710936a.
- [86] K. L. Mueller, "Recognizing the first responders," *Science*, vol. 327, no. 5963, p. 283, 2010, doi: 10.1126/science.327.5963.283.
- [87] M. C. Matesanz *et al.*, "Early in vitro response of macrophages and T lymphocytes to nanocrystalline hydroxyapatites," *J. Colloid Interface Sci.*, vol. 416, pp. 59–66, Feb. 2014, doi: 10.1016/j.jcis.2013.10.045.
- [88] S. Tai *et al.*, "Characterization of beta-tricalcium phosphate as a novel immunomodulator," *Int. Immunopharmacol.*, vol. 19, no. 1, pp. 45–51, 2014, doi: 10.1016/j.intimp.2013.12.024.
- [89] S. R. Krutzik *et al.*, "TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells," *Nat. Med.*, vol. 11, no. 6, pp. 653–660, 2005, doi: 10.1038/nm1246.
- [90] Y. H. Luo, L. W. Chang, and P. Lin, "Metal-Based Nanoparticles and the Immune System: Activation, Inflammation, and Potential Applications," *BioMed Res. Int.*, vol. 2015, no. Figure 1, 2015, doi: 10.1155/2015/143720.
- [91] V. Sokolova, T. Knuschke, A. Kovtun, J. Buer, M. Epple, and A. M. Westendorf, "The use of calcium phosphate nanoparticles encapsulating Toll-like receptor ligands and the antigen hemagglutinin to induce dendritic cell maturation and T cell activation," *Biomaterials*, vol. 31, no. 21, pp. 5627–5633, 2010, doi: 10.1016/j.biomaterials.2010.03.067.
- [92] D. Kozlova *et al.*, "Calcium phosphate nanoparticles show an effective activation of the innate immune response in vitro and in vivo after functionalization with flagellin," *Virol. Sin.*, vol. 29, no. 1, pp. 33–39, 2014, doi: 10.1007/s12250-014-3379-0.
- [93] F. Hayashi *et al.*, "The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5," *Nature*, vol. 410, no. 6832, pp. 1099–1103, 2001, doi: 10.1038/35074106.
- [94] T. Behera and P. Swain, "Antigen adsorbed calcium phosphate nanoparticles stimulate both innate and adaptive immune response in fish, *Labeo rohita* H.," vol. 271, pp. 350–359, 2011, doi: 10.1016/j.cellimm.2011.07.015.
- [95] R. Medzhitov, "Recognition of microorganisms and activation of the immune response," *Nature*, vol. 449, no. 7164, Art. no. 7164, Oct. 2007, doi: 10.1038/nature06246.
- [96] Q. He, A. R. Mitchell, S. L. Johnson, C. Wagner-Bartak, T. Morcol, and S. J. D. Bell, "Calcium phosphate nanoparticle adjuvant," *Clin. Diagn. Lab. Immunol.*, vol. 7, no. 6, pp. 899–903, 2000, doi: 10.1128/CDLI.7.6.899-903.2000.



- [97] K. Viswanathan, V. P. Gopinath, and G. D. Raj, "Formulation of Newcastle disease virus coupled calcium phosphate nanoparticles: An effective strategy for oculonasal delivery to chicken," *Colloids Surf. B Biointerfaces*, vol. 116, pp. 9–16, 2014, doi: 10.1016/j.colsurfb.2013.12.017.
- [98] D. H. Joyappa, C. Ashok Kumar, N. Banumathi, G. R. Reddy, and V. V. S. Suryanarayana, "Calcium phosphate nanoparticle prepared with foot and mouth disease virus P1-3CD gene construct protects mice and guinea pigs against the challenge virus," *Vet. Microbiol.*, vol. 139, no. 1–2, pp. 58–66, 2009, doi: 10.1016/j.vetmic.2009.05.004.
- [99] V. V. Temchura, D. Kozlova, V. Sokolova, K. Überla, and M. Epple, "Targeting and activation of antigen-specific B-cells by calcium phosphate nanoparticles loaded with protein antigen," *Biomaterials*, vol. 35, no. 23, pp. 6098–6105, 2014, doi: 10.1016/j.biomaterials.2014.04.010.
- [100] B. Y. Chua, T. Sekiya, M. Al, K. R. Short, D. E. Mainwaring, and D. C. Jackson, "Biomaterials A single dose biodegradable vaccine depot that induces persistently high levels of antibody over a year," *Biomaterials*, vol. 53, pp. 50–57, 2015, doi: 10.1016/j.biomaterials.2015.02.066.
- [101] C. Zilker *et al.*, "Nanoparticle-based B-cell targeting vaccines: Tailoring of humoral immune responses by functionalization with different TLR-ligands," *Nanomedicine Nanotechnol. Biol. Med.*, vol. 13, no. 1, pp. 173–182, 2017, doi: 10.1016/j.nano.2016.08.028.
- [102] Z. Sadeghi, M. Fasihi-ramandi, and S. Bouzari, "Nanoparticle-Based Vaccines for Brucellosis: Calcium Phosphate Nanoparticles-Adsorbed Antigens Induce Cross Protective Response in Mice," pp. 3877–3886, 2020.
- [103] S. Dodangeh, M. Fasihi-ramandi, A. Daryani, and R. Valadan, "Microbial Pathogenesis Protective efficacy by a novel multi-epitope vaccine , including MIC3 , ROP8 , and SAG1 , against acute *Toxoplasma gondii* infection in BALB / c mice," *Microb. Pathog.*, vol. 153, no. January, p. 104764, 2021, doi: 10.1016/j.micpath.2021.104764.
- [104] T. J. Levingstone, S. Herbaj, and N. J. Dunne, "Calcium Phosphate Nanoparticles for Therapeutic Applications in Bone Regeneration," *Nanomaterials*, vol. 9, no. 11, Art. no. 11, Nov. 2019, doi: 10.3390/nano9111570.
- [105] D. Huang, B. He, and P. Mi, "Calcium phosphate nanocarriers for drug delivery to tumors: imaging, therapy and theranostics," *Biomater. Sci.*, vol. 7, no. 10, pp. 3942–3960, Sep. 2019, doi: 10.1039/C9BM00831D.
- [106] R. Khalifehzadeh and H. Arami, "Biodegradable calcium phosphate nanoparticles for cancer therapy," *Adv. Colloid Interface Sci.*, vol. 279, p. 102157, May 2020, doi: 10.1016/j.cis.2020.102157.
- [107] S. Chandrasekaran and M. R. King, "Microenvironment of Tumor-Draining Lymph Nodes: Opportunities for Liposome-Based Targeted Therapy," *Int. J. Mol. Sci.*, vol. 15, no. 11, Art. no. 11, Nov. 2014, doi: 10.3390/ijms151120209.
- [108] C. J. M. Melief, T. van Hall, R. Arens, F. Ossendorp, and S. H. van der Burg, "Therapeutic cancer vaccines," *J. Clin. Invest.*, vol. 125, no. 9, pp. 3401–3412, Sep. 2015, doi: 10.1172/JCI80009.
- [109] Z. Y. Xu-Monette, M. Zhang, J. Li, and K. H. Young, "PD-1/PD-L1 Blockade: Have We Found the Key to Unleash the Antitumor Immune Response?," *Front. Immunol.*, vol. 8, p. 1597, Dec. 2017, doi: 10.3389/fimmu.2017.01597.
- [110] M. W. L. Teng, S. F. Ngiow, A. Ribas, and M. J. Smyth, "Classifying Cancers Based on T-cell Infiltration and PD-L1," *Cancer Res.*, vol. 75, no. 11, pp. 2139–2145, Jun. 2015, doi: 10.1158/0008-5472.CAN-15-0255.
- [111] J. L. Geiger, J. R. Grandis, and J. E. Bauman, "The STAT3 pathway as a therapeutic target in head and neck cancer: Barriers and innovations," *Oral Oncol.*, vol. 56, pp. 84–92, May 2016, doi: 10.1016/j.oraloncology.2015.11.022.
- [112] S. Du Four *et al.*, "Axitinib increases the infiltration of immune cells and reduces the suppressive capacity of monocytic MDSCs in an intracranial mouse melanoma model," *Oncimmunology*, vol. 4, no. 4, p. e998107, Apr. 2015, doi: 10.1080/2162402X.2014.998107.
- [113] C. T. Garnett, J. Schlom, and J. W. Hodge, "Combination of Docetaxel and Recombinant Vaccine Enhances T-Cell Responses and Antitumor Activity," *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.*,
- [114] J. W. Hodge, A. Ardiani, B. Farsaci, A. R. Kwilas, and S. Gameiro, "The Tipping Point for Combination Therapy: Cancer Vaccines with Radiation, Chemotherapy, or Targeted Small Molecule Inhibitors," *Semin. Oncol.*, vol. 39, no. 3, pp. 323–339, Jun. 2012, doi: 10.1053/j.seminoncol.2012.02.006.