Advanced Nanobiomaterials: Vaccines, Diagnosis and Treatment of Infectious Diseases

Eva Torres-Sangiao 1, Alina Maria Holban 2-3 and Monica Cartelle Gestal 4,*

1 Department of Microbiology and Parasitology, University Santiago de Compostela, Galicia 15782, Spain; eva.torres.sangiao@gmail.com
2 Department of Microbiology and Immunology, Faculty of Biology, University of Bucharest, Bucharest 060101, Romania; alina_m_h@yahoo.com
3 Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, Bucharest 060042, Romania
4 Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens (UGA), GA 30602, USA
* Correspondence: mcarges@gmail.com or mcgestal@uga.edu; Tel.: +1-706-202-5304

Academic Editors: Ecaterina Andronescu and Alexandru Mihai Grumezescu

Received: 25 May 2016; Accepted: 25 June 2016; Published: 1 July 2016

Abstract: The use of nanoparticles has contributed to many advances due to their important properties such as, size, shape or biocompatibility. The use of nanotechnology in medicine has great potential, especially in medical microbiology. Promising data show the possibility of shaping immune responses and fighting severe infections using synthetic materials. Different studies have suggested that the addition of synthetic nanoparticles in vaccines and immunotherapy will have a great impact on public health. On the other hand, antibiotic resistance is one of the major concerns worldwide; a recent report of the World Health Organization (WHO) states that antibiotic resistance could cause 300 million deaths by 2050. Nanomedicine offers an innovative tool for combating the high rates of resistance that we are fighting nowadays, by the development of both alternative therapeutic and prophylaxis approaches and also novel diagnosis methods. Early detection of infectious diseases is the key to a successful treatment and the new developed applications based on nanotechnology offer an increased sensibility and efficiency of the diagnosis. The aim of this review is to reveal and discuss the main advances made on the science of nanomaterials for the prevention, diagnosis and treatment of infectious diseases. Highlighting innovative approaches utilized to: (i) increasing the efficiency of vaccines; (ii) obtaining shuttle systems that require lower antibiotic concentrations; (iii) developing coating devices that inhibit microbial colonization and biofilm formation.

Keywords: nanoparticles; vaccines; microbiology diagnosis; biofilm; antibiotic resistance

1. Introduction

In 1959, Richard Feynman described a process that allows one to individually manipulate atoms and molecules throughout high precision instruments. This system could be applied to design and build systems at nanoscale level, atom by atom [1,2] and its applications in many areas of wide interest such as health, industry, pharmacy, etc., seem to be unlimited. In 1981 the engineer Eric Drexler, inspired by Feynman’s speech, published the article entitled “Molecular engineering: An approach to the development of the general capabilities for molecular manipulation” in which he described more in detail what Feynman have previously described [3]. The term “nanotechnology” was first applied by Drexler in 1986 [4] and it has been used for this area of expertise since.
Nanotechnology refers to the area of the knowledge that designs and produces structures, devices and systems by manipulating atoms and molecules at the nanoscale level [5]. Nanoparticles are microscopic particles smaller than 100 nanometers [6]. Due to their small size, nanoparticles have unusual properties which make their use in nanomedicine advantageous [7]. Nowadays most nanoparticles are obtained from transition metals, silicon, carbon and metal oxides.

Nanobiotechnology is the area of nanotechnology focused on the biological field. Nanoparticles utilized in biology are grouped into three categories: organic, inorganic and mixed (organic/inorganic) [8]. In recent years, many nanoparticles have been developed for diverse applications in medicine, including infectious diseases. The development of nanoparticles in this area has been beneficial due to their selective antimicrobial effect with low toxicity against the host and their ability to place their action on specific targets.

Shuttle systems are commonly used for the delivery and stabilization of bioactive drugs and antimicrobial molecules, ensuring not just their specificity but also controlled release [8]. Coating medical devices is of a great advantage in the infectious disease field, e.g., nanomodified surfaces and devices proved to be very efficient to reduce microbial attachment and biofilm formation [9,10].

The use of nanomaterials as biosensors has currently a vast impact and a fast development in the usage of smart nanobiomaterials. Biosensors are accurate and offer a cost effective approach for the detection of pathogenic infectious agents in natural environment, food but also clinical specimens [11]. Nanodiagnostics was first introduced by Mirkin et al. [12] in 1996 where the authors published the use of gold (Au)-nanoparticles to allow anthrax detection [13].

The aim of this review is to highlight and discuss the recent progress and applications of nanotechnology in the medical field (nanomedicine) focusing on the prevention, diagnosis and treatment of infectious diseases.

2. Nanoparticles and Vaccines

Traditional vaccines have been developed using live attenuated organisms (cellular vaccines) or inactivated toxins or proteins (acellular vaccines). Recently, the development of synthetic peptide-based vaccines has shown many advantages compared with traditional vaccines, such as better safety and/or conservation. However, the peptide-based vaccines generate a weaker immune response, and the inclusion of adjuvants and/or the use of vaccine delivery systems is highly needed [14]. Antibacterial vaccines, both cellular and acellular, are still considered the most cost effective intervention against bacterial infections. Implementation of vaccine schedules has decreased worldwide the morbidity and mortality caused by infectious diseases such as diphtheria, pneumococcal and pertussis diseases. However, the treatment and prevention of other common bacterial infections, including but not limited to Staphylococcus aureus, Helicobacter pylori, Shigella spp. or Escherichia coli is still not possible [15].

Nanoparticles have several applications in nanobiomedicine, especially in the field of vaccine production where they can be applied as efficient delivery systems. Their particular nature increases cross-presentation of the peptide [16] and it also plays an important role in the activity of antigen presentation cells (APC) [16,17]. The main application of synthetic nanoparticles in immune engineering relies on the modulation of APC, by encapsulating or releasing molecules that promote dendritic cell activation, triggering particle-specific immune recognition and thus, antigen processing. Nanoparticles can further act as co-adjuvants, stimulating the proper pro- or anti-immunity pathways. This immuno-stimulation can be achieved by encapsulation of various compounds and/or according to their structure or composition [17]. In addition, hypersensitivity produced by the nanoparticles used can be ameliorated by slowing the rate of infusion of the delivery nanovaccine system, by modulating their shape and size, or by patient premedication [18].

Nanomaterials may have intrinsic immunomodulatory functions, acting as adjuvants or immune potentiators [17]. According to the nanomaterial composition [19], the vaccine-associated nanoparticles [20] could be classified in different types (Table 1).
(i) Polymers, divided in turn into nanoparticles containing synthetic polymers, such as poly(D,L-lactic-co-glycolic acid) (PLGA) [21], polyethylene glycol (PGE) [22] or polyester bio-beads [23], and natural polymers based on polysaccharides such as alginate [24], inulin [25] or chitosan [26]. Synthetic and natural polymers have been used to synthesize hydrogel nanoparticles, which have favorable properties including but not limited to flexible mesh size, large surface area for multivalent conjugation, high water content, and high antigen loading capacity [27];

(ii) Liposomes, which are biodegradable and non-toxic phospholipids. They encapsulate antigens and incorporate viral envelope glycoproteins to form virosomes. The combination of a modified cationic liposome and a cationic polymer (such as protamine)-condensed DNA is called liposome-polycation-DNA nanoparticles (LPD). They are commonly used as adjuvant delivery system in DNA vaccine studies [28];

(iii) Nanosized emulsions are those nanoparticles that can exist as oil-in-water or water-in-oil form. Emulsions can carry antigens inside their core to increase the efficiency of vaccine delivery or they can also be simply mixed with antigen [20];

(iv) Inorganic nanoparticles are non-biodegradable, they have rigid structure and controllable synthesis. Silica-based nanoparticles (SiNPs) offer the advantage of biocompatibility and have excellent properties as nanocarriers. SiNPs particles such as mesoporous silica nanoparticles (MSNs) could potentially become high-efficient, controlled-release nanocarriers in future vaccine formulations [20].

(v) Immuno-stimulating complexes (ISCOM). They are composed of supra-molecular structures of the adjuvant Quil A and immunizing peptides, which allows selective incorporation of viral envelope proteins by hydrophobic interaction [29].

(vi) Virus-like particles (VLP) are optimized for interaction with the immune system, avoiding the infectious components. They can induce potent immune responses, even in the absence of adjuvant [30]. VLP based vaccines have been the first nanoparticle class to reach market [31], found for example under the following Engerix® RECOMBIVAX® HB against to HBV [32].

(vii) Self-assembling systems emerged as a consequence of an attempt to drive higher levels of protein, and consequently better immunological properties. A variety of natural proteins can be self-assembled into nanoparticles, conferring highly symmetric, stable and organized structure [32].
**Table 1.** Most representative vaccine applications based on nanotechnology.

| Type of Nanoparticles | Based on | Main Characteristic | Use | Representative Uses | Ref. |
|-----------------------|----------|---------------------|-----|---------------------|-----|
| **Polymers**          |          |                     |     |                     |     |
| PLG                   |          |                     |     | Toxoplasmosis       |      |
| PLGA                  | Biocompatibility & biodegradability | entrap antigen for delivery (carrier) to certain cells and sustain Ag release according to their biodegradation rate |   | S. aureus           | [35] |
|                       |          |                     |     | TB                  | [36] |
|                       |          |                     |     | Brucella abortus    |      |
|                       |          |                     |     | Anthrax             | [20] |
|                       |          |                     |     | Plasmodium vivax    |      |
|                       |          |                     |     | HBV                 |      |
| PGE                   | Can be conjugated with a variety of Ag or surface-modified with various functional groups | |   | Influenza Virus     | [38] |
| Polystyrene           |          |                     |     | HIV                 | [39] |
|                       |          |                     |     | P. malariae         | [40] |
| Polyester Bio-Beds    | Vaccine delivery system low cost & biocompatibility | |   | TB                  | [41] |
| **Inulin: ADVAX™**    | Activator of complement alternative pathway, potent adjuvant. | |   | Antrax              | [42] |
|                       |          |                     |     | Listeria monocytogenes | [43] |
|                       |          |                     |     | Influenza virus     | [44] |
|                       |          |                     |     | SARS-CoV            | [45] |
|                       |          |                     |     | HBV                 | [25] |
|                       |          |                     |     | HIV, JVE-WNV        | [46] |
| Alginate              | Biocompatibility, biodegradability & nontoxic adjuvant | |   | K. pneumoniae       | [47] |
|                       |          |                     |     | P. aeruginosa       | [48] |
| Pullulan              |          |                     |     | Influenza virus     | [49] |
|                       |          |                     |     | Difteria            | [50] |
|                       |          |                     |     | E. coli O157:H7     | [51] |
|                       |          |                     |     | P. aeruginosa       | [52] |
|                       |          |                     |     | Influenza virus     | [38, |
|                       |          |                     |     | HBV                 | [53] |
|                       |          |                     |     | Filariasis          | [54] |
|                       |          |                     |     | Dengue              | [55] |
|                       |          |                     |     | S. pneumoniae       | [57] |
| Chitosan              | Easily modified | |   | C. botulinum        | [20] |
| Hydrogel              | Flexible mesh size | |     | S. pneumonia       | [58] |
|                       | Large surface area for multivalent conjugation: high capacity for Ag | |   | Papilomavirus       | [59] |
|                       | Hydrophilic 3D polymer network. | |   | NDV                 | [60] |
| Pullulan              |          |                     |     | Dengue              | [61] |
| Type of Nanoparticles | Based on | Main Characteristic | Use | Representative Uses | Ref. |
|-----------------------|----------|---------------------|-----|---------------------|-----|
| **Liposomes**         | LPD      | phospholipids       | Biodegradable & nontoxic encapsulate Ag and form virosomes | adjuvant | P. malarie, Influenza Virus (INFLLEXAL® V), HAV (Epaxal®), HIV | [40][62][63] |
|                       |          |                     |     |                     |     |
|                       | ICMV     |                     | adjuvant carrier | P. vivax | [40] |
| **Emulsions**         | Oil-in-water/water-in-oil | MF59™ | Mixed with Ag & transport | adjuvant | Influenza Virus (FLUAD©, AFLUNOV©, FOCETRIA®, OPTAFLU©), HAV (Epaxal®) | [62] |
|                       |          |                     |     |                     |     |
|                       | AS03/AS04 |                     |     |                     |     |
|                       | Montanide™ |                  |     |                     |     |
| **Inorganic**         | AuNP     | Au/gold             | APC cytokine production can be induced according to shape and size | Adjuvant recognition, absorption of specific biomolecules, improvement of interaction with cells & enhancement of cellular uptake | Pneumococci, L. monocyctogenes, Burkholderia mallei, Yersinias pestis, P. falciparum | [68][43][60][70][71] |
|                       |          |                     |     |                     |     |
|                       | CNT      | Carbon              | Good biocompatibility, Synthesized into a variety of nanotubes and mesoporous spheres multiple copies of protein and peptide Ag | HIV | P. vivax, E. coli 0111, Influenza Virus, HBV | [73][74][75][76] |
|                       | SiNP     | Si                  | Biocompatible | | Enterovirus 71, NDV, HIV | [77][78][79] |
|                       | calcium  | Ca                  | Excellent biocompatibility & non-toxic for DNA vaccines and mucosal immunity | | | |
### Table 1. Cont.

| Type of Nanoparticles | Based on | Main Characteristic | Use | Representative Uses | Ref. |
|-----------------------|----------|---------------------|-----|---------------------|-----|
| ISCOM                 | Quil A, cholesterol, phospholipids & protein Ag | Trap the Ag by apolar interactions mucosal immunity | Adjuvant | S. aureus, P. malarie, Chaagas disease, Tetanus, Influenza Virus, HSV, HBV, HIV | [80], [40], [81], [82], [20] |
| VLP                   | Self-assembly biocompatible capsid protein | Evolved viral structure & delivery platform | Induce potent immune responses | Papilomavirus (Cervarix®, GARDA SIL®), HBV (Engerix®, RECOMBIVAX®HB), HIV, Influenza Virus, Marburg, Ebola, E. coli, P. falciparum, Norovirus, HEV (Hecolin), VZV, HCV, Enterovirus, Chikungunya Virus, S. pneumoniae | [62], [83], [32], [84], [85], [86], [87], [88] |
| ferritin              | Fe       | Attempt to drive higher levels of protein quaternary structuring | Adjuvant | Influenza Virus, VEB, HCV, HIV | [32] |
| MVP                   | Protein  |                       | Adjuvant | HIV | [32] |
| Self-assembling systems | SAPNs    | Peptides | Ability to repetitively present Ag Better biophysical & immunological properties | Strong immunogenic effect (of cellular vaccines) Purity & high specificity immune responses | P. malarie, Toxoplasmosis, Influenza Virus, HIV, HCV | [90], [91], [92], [93] |

Ag: antigens; AuNP: Gold nanoparticles; GNP: gold glyco-NPs; HAV: Hepatitis A Virus; HEV: Hepatitis E Virus; HSV: Herpes Simple Virus; ICMVs: Liposomes modified with maleimide synthesized into interbilayer-crosslinked multimamellar vesicles; ISCOM: Immuno-stimulating complex; JEV: Japanese encephalitis virus; LPD: liposome-polycation-DNA nanoparticles; MSNs: mesoporous silica nanoparticles; MVP: vault protein; NDV: Newcastle disease virus; PGE: poly(ethylene glycol); PLG: poly(ε-caprolactone-co-glycolic acid); PLGA: poly(ε-caprolactone-co-glycolic acid); SAPN: self-assembling peptide nanoparticles; SARS-CoV: Severe acute respiratory syndrome-associated coronavirus; VEB: Virus Epstein-Bar; VZV: Varizela Zoster Virus; WNV: West Nile virus.
3. Microbial Detection by Using Nanoparticles

The emergence of infections together with the fast evolution of drug-resistant bacteria (superbugs), are triggering the increased ineffectiveness of actual therapies used to treat infectious diseases [94]. Clinical microbiology laboratories still use the conventional phenotypic methods for the identification of bacteria and novel mechanisms of resistance. Nowadays laboratories are supported by molecular biology techniques, such as those based on 16S rRNA sequencing, but also various advanced physico-chemical analysis. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is becoming a reliable method for microbial identification in several hospitals, due to their speed, accuracy and cost effectiveness [95]. For the design of an optimal diagnosis method, some parameters should be considered: this would be a cost-effective, portable, and point of source-detection system which would be also highly reliable, sensitive, and accurate [96]. The desirable method also should be able to detect multiple pathogens in one single run.

A number of nanotechnology-based materials have been studied with the purpose of controlling and preventing infectious diseases [97]. The physical and chemical properties of nanoparticles allow accurate, rapid, sensitive, and cost-efficient diagnostics [94]. Antibody-based diagnoses such as those utilizing Fluorescent Silica Nanoparticles (FSNPs) have been developed in order to detect Mycobacterium tuberculosis complex (MTB) within 4 h [98]. Incorporating europium [Eu(III)] polymeric nanoparticles have been successfully for the detection of anthrax antibodies by using fluorescence enzyme linked immunosorbent assay (ELISA) [94]. A combination of positive di-electrophoresis and aptamer-FSNPs label has been developed as a rapid and sensitive method for detection of S. aureus [99].

Wang and Kang [100] have developed recently, a method for detection of Salmonella typhimurium based on a single-stranded DNA aptamers along with silica fluorescence nanoparticles. Liposomes can recognize target toxins, and therefore they can be used for toxin detection. Liposomes labelled with fluorescent markers (such as rhodamine dyes) can be incorporated into sandwich fluoro-immunoassay on antibody-coated microtiter plates in order to detect toxins [101]. Ahn-Yoon et al [102], had used this method to detect cholera toxin within a limit of detection of 10 fg/mL and in only 20 min [102]. A similar assay was developed to detect botulinum toxin (BT) on a nitrocellulose membrane strip by using tri-sialo-ganglioside GT1b-liposomes, which is a receptor for BT [103].

Quantum dots (QDs) are special nanocrystalline semiconductors [94] composed of materials such as ZnS, ZnSe, CdS, CdSe, CdTe and InP, among others [104]. QDs show strong resistance to photobleaching and chemical degradation, as well as significant photostability and high quantum yield [105]. These characteristics make them suitable for sensitive image acquisition and signal amplification in real time [94]. The applications of QDs in nanobiomedicine are diverse, varying from fluorescent probes, biosensors to therapeutics agents [104]. Numerous methods have been developed for creating hydrophilic QDs [106]. Among QDs’ bioapplications, it is important to highlight, multiplex detection of analytes based in single molecule detection. QD-based nanosensors are an example of a highly sensitive, extremely low cost-per-sample technique, that ensures short analysis time and it has the potential to be applied for rapid detection of viral and bacterial proteins, with enhanced sensitivity and specificity over conventional organic fluorophores [104].

In 2010, Zhang and Hu [107] developed a multiplex assay for the detection of HIV-1 and HIV-2. This single-QD-based nano-sensor showed an extremely low sample consumption, high sensitivity and short analysis time. These results have shown the many advantages of this method to be applied for rapid point-of-care testing, gene expression studies, high-throughput screening, and clinical diagnostics. Six years later, Zhang et al. [108] designed an efficient immunosensor-based technique for screening and isolating Salmonella sp. with a detection limit of 10 cell/mL. The aforementioned fluorescent nanobioprobes made on a specially designed cellulose-based swab could be applied in a large number of samples related to public health surveillance to visually detect and directly isolate pathogens in situ.
In 2007, Klostranec et al. [109] reported the use of QDs with microfluidics for the obtention of bio-imaging signals, improving the high sensitivity for their use in diagnosis. QD-antibody conjugates has also been successfully used in fluoro-immunoassays for the detection of staphylococcal enterotoxin B [110], syncytial respiratory virus [111] or hepatovirus, and HVB, HCV, and HIV viruses [112]. Ebrahim et al. [113] have been able to synthesize CdTe-QDs conjugated with concanavalin A for the detection of lipopolysaccharide (LPS) produced by *Serratia marcescens* with a detection range from 10 to 10^6 colony forming units/mL (CFU/mL) at pH 7.

Detection systems based on noble metal nanoparticles (Table 2), particularly Au and Ag, have been widely studied due to their unique optical and physicochemical properties [114] and they are known as surface plasmon resonance (SPR) [94]. Their nano-size scale and their optical/physicochemical properties have been used for selective and specific identification of DNA/RNA sequences, proteins, or small analytes associated with the presence of infection and various pathogens. Their detection relies on colorimetric assays, fluorescence, mass spectrometry, electrochemical, and scattering approaches [95]. In 2005, Duan et al. [115], reported the usage of immune-gold silver staining with Au-nanoparticles as a very sensitive method for the detection of single molecules and its application for the detection of HCV and HBV.

**Magnetic nanoparticles** (MNPs) have nanoscale sizes, which mimic the size of molecules in nature, and they harbor favorable characteristics for their use in nano-biomedicine, such as imaging and therapy [105]. Surface modification of MNPs with recognition moieties, for instance, antibodies, antibiotics, and carbohydrate, enables their use for bacterial detection.

These recognition moieties help to detect the bacteria selectively and at low concentrations [94,116]. The super-paramagnetic property provides MNPS with a promising and sophisticated platform for in vivo detection techniques and have the potential to make microbiological diagnostics become much easier and more worthy [116,117] (Table 2). MNPs can be classified as metal, alloys or oxides, and are generally based on elements such as Fe, Co, Ni, or Mn, among others [105]. Iron oxide nanoparticles (IONPs) are the most studied and are composed of magnetite (Fe₃O₄) or maghemite (γ-Fe₂O₃) nanocrystallites. IONP-biosensors have been developed for the detection of HSV-1 and adenoviruses enabling to detect five viral particles in 10 µL serum samples without previous PCR amplification steps [118]. Using IONPs functionalized with IgG [119] and vancomycin [120] have allowed to push the limit of detection to 10⁴ CFU/mL bacterial cells by using nano-MALDI platforms [121]. Nanodiagnostic systems will allow microbiologists to perform molecular tests faster and with higher sensitivity. These methods also increase flexibility at reduced costs [122]. However the majority of these new nanoplatfoms still need further evaluation and validation with clinical samples before they can be fully translated into clinical diagnosis.
### Table 2. Microbiological diagnosis approach by inorganic nanoparticles.

| Nanoparticle               | Based on                                                                 | Detection/Identification by                      | Detection | Limit of Detection | Ref.  |
|----------------------------|--------------------------------------------------------------------------|---------------------------------------------------|-----------|--------------------|-------|
| **AgNPs**                  | Label-free near infrared surface-enhanced Raman scattering (NIR-SERS)    | MRSA, *Listeria* spp., *E. coli* & *P. aeruginosa* |           | $10^3$ CFU/mL      | [123] |
| **Au-coted-NPs**           | Surface-enhanced Raman scattering spectroscopy (SERS)                    | Spectrum                                          | Legionella spp. |                   | [124] |
| **Vancomycin coated Ag-Au-NPs** | Surface-enhanced Raman scattering spectroscopy (SERS)                   | *S. epidermidis*, *B. megaterium*, *E. coli* & *Salmonella enterica* | $10^5$ CFU/mL |                   | [125] |
| **AuNP**                   | Differential stabilization of Au-nanoprobes in presence of DNA targets following salt induced aggregation | Colorimetric detection from red to blue           | TB         | $0.75 \ \mu g/2 \ h$ | [11]  |
| **Cross-linking**          | Cross-linking approach, where the target DNA acts as a linker between two different Au nanoprobes, based on auper aggregation | Colorimetric detection from red to blue           | MRSA      | $66 \ \mu g/\mu L (<10^6 \ CFU/mL)$ | [11]  |
| **Interaction aups–dsdna & the addition of thiolated probes specific to the inva gene in the *Salmonella* genomic DNA aggregates aups** | Colorimetric detection from red to violet | *S. enterica* | 37 fM        | [127] |
| **The ability of ssdna oligo-targeters to stabilize the colloidal aups preventing their salt-induced aggregation.** | Colorimetric detection | *Acinetobacter baumanii* | 0.8125 ng/\mu L | [128] |
| **Non-cross-linking**      | Non-cross-linking method results from the differential aggregation profiles of Au-nanoprobes induced by increased ionic strength in the presence or absence of the specific target sequence | Colorimetric detection (SPR band: 525–650 nm) | MTBC and *Plasmodium* | [129] |
| **Multichannel fluorescence sensor** | Ratiometric response according to three-color RGB output | BIOFILMS: *Amycolatopsis azurea*, *B. lichenformic*, *B. megaterium*, *E. coli*, *P. aeruginosa* | [131] |
| **Fast lateral flow immunoassay (FLFI) approach combined with rapid “one step” lysis** | Colorimetric detection | *E. coli* | $5 \times 10^4 \ \text{CFU/mL}$ 25 min | [132] |
| **Aptamer-conjugated-AuNPs** | aptamer–DNA duplex formed by the hybridization reaction between the capture probe and the aptamer probe, which induces a clear enzymatic catalysis of the oxidation of methionine by hydrogen peroxide | Biosensor | *C. difficile* | 1 nM | [133] |
| **glassy carbon electrode modified with graphene oxide and AuNPs** | Electrochemical impedance spectrum | *Salmonella* | 3 CFU/mL | [134] |
| **cell-based SELEX (Systematic Evolution of Ligands by Exponential Enrichment), and dissociation constants and binding specificity** | Resonance light-scattering–detection system | *S. aureus* | 10 CFU/mL 1.5 h | [135] |
| **AuNPs paper-paper**     | Non-cross-linking assay wax-printed microplate paper platform            | Colorimetric detection from red to blue           | TB         | $30 \ \mu g/mL$ 2 h | [136] |
| Nanoparticle | Based on | Detection/Identification by | Detection | Limit of Detection | Ref. |
|--------------|----------|-----------------------------|-----------|-------------------|-----|
| **Microarrays** | | | | | |
| AuNPs | Multiple capture and intermediate oligos to detect a target in multiple regions | Silver signal by scannomatic detection | Influenza Virus H5N1 | <10⁷ copies of transcribed RNA, 2.5 h | [137] |
| Ag-Au core shell NPs | Nanoparticle-based microarrays using a photodiode sensor | SEM images | HPV | 0.05 pmol/µL | [138] |
| **Magnetic nps** | | | | | |
| AuMNPs | Non cross-linking aggregation phenomenon: specific interaction between mecA gene with the gold | Colorimetric detection (λ = 530 nm) | MRSA | | [139] |
| | Electrochemical geno-sensing assay onto the latex microspheres | AuNPs signal | Vibrio cholerae | 2 CFU/mL | [140] |
| Fept@Vanco | Trapping gram-positive bacteria, based on interaction between the heptapeptide backbone of vancomycin and the D-alanyl-D-alanine dipeptide from the cell wall | MALDI-TOF | Staphylococcus spp., VRE & E. coli | 100 CFU/mL | [116] |
| **Imunoassay** | | | | | |
| Au-NPs | AuNPs bound to anti-human IgG | Colorimetric immunoassay | Influenza Virus | 10 pg/mL | [141] |
| | FLFI combined with ELISA | RAMAN Intensity | E. coli 0157:H7 | 10⁵ CFU/mL | [142] |
| | Plasmonic ELISA (ELISA with enzyme-mediated SPR of AuNPs) | Chemiluminescence | Salmonella spp. | 50–100 CFU/mL | [145] |
| Ag/NPs | SERS enzyme-catalyzed immunoassay | Fluorescence signal | HIV-1 p24 | <0.1 pg/mL | [146] |
| EutIII-NPs | ELISA, antigen-antibody immunoreaction | Fluorescence signal | | | |
| AgNP-G | gold electrode coated with AuNP-G, whose is modified with H7-monoclonal antibodies | Electrochemical immunosensor | Aviar Influenza Virus H7 | 1.6 pg/mL | [147] |
| FSNPs | highly fluorescent bioconjugated nanoparticles probe | Fluorescence signal | L. monocytogenes | 50 CFU/mL | [148] |
| **Fluorescence** | | | | | |
| Si-MNPs | high specificity for dsDNA and bright fluorescence upon intercalation into dsDNA | Nucleic-acid dye SYBR Green I signal (Intensity) | S. aureus | 50 CFU/mL | [149] |

AgNPs-G: silver nanoparticle-graphene; HPV: Human Papiloma Virus; FLFI: fast lateral flow immunoassay; MDRTB: Multi Drug resistance TB; MRSA: Methicillin resistant S. aureus; MTB: multidrug resistant TB; MTBC: Micobacterium tuberculosis complex; SERS: Surface-enhanced Raman scattering spectroscopy; SPR: Surface Plasmon resonance; SRV: Syncytial Respiratory Virus; TB: Tuberculosis; VRE: Vancomycyn Resistant Enterococcus spp.
4. Nanoparticles for Fighting Superbugs

Drug resistance is of a great concern for public health. The use of high dose antibiotic treatments often generates high rates of toxicity and the development of new resistance. In addition, the costs of treatments increase while there is a major number of treatment failures and high spectrum therapies associated with an increase in the number of hospitalization days. Due to the lack of new alternatives for the treatment of infectious diseases, several classes of antimicrobial nanoparticles and nanocarriers for antibiotic delivery have been studied, as well as their effectiveness for the treatment of infectious diseases, including antibiotic resistant bacteria [150].

Nanoparticles provide a versatile platform for the design of materials with antimicrobial properties. Their unique nanoscale as well as physical and chemical properties provide multiple attributes that facilitate the development of unique antimicrobial strategies; hence, they are emerging as weapons in our antimicrobial arsenal. These nano-antimicrobial materials can be synthesized by variety of different methods influencing subsequent antimicrobial effect [151]. They could be divided into inorganic, organic and hybrid nanoparticles. The most advantageous are inorganic nanomaterials, such as Ag and Au, alone or combined with various organic polymers (Figure 1).
Molecules 2016, 21, 867

Figure 1. Representative uses of the main nanoparticles developed for the treatment of infectious diseases. Abbreviations: CNTs: Carbon nanotubtes; ESBLs: expanded spectrum beta lactamases; GAS: Streptococcus group A (S. pyogenes); GBS: Streptococcus group B (S. agalactiae); HPV: human papilloma virus; HSV: herpes simplex virus; IRP: Isoniazide-rifampicin-paramycin; MDR: multi-drug-resistance; MRSA: methicillin resistance S. aureus; MRSE: methicillin resistant S. epidermidis; NPs: nanoparticle; PLGA: poly(l,l-lactic-co-glycolic acid); SRV: syncital respiratory virus; TB: tuberculosis (Mycobacterium tuberculosis); VRSA: vancomycin resistant S. aureus.

The antimicrobial mechanism of the action of nanoparticles is not fully known. Nevertheless, the antimicrobial actions include destruction of cell membranes, blockage of enzyme pathways, alterations of microbial cell wall, and nucleic materials pathway. The applicability of nanoparticles as therapeutic agents includes a wide range of action, varying from broad spectrum antimicrobial agents, sterilization and wound healing agents, to sustained inhibitors of intracellular pathogens [152]. The most of the tested nanoparticles are highly efficient against Staphylococcus aureus and Escherichia coli, and according to properties they have been even used to treat tuberculosis (TB) [36] (Figure 1).

The antibacterial activity of Ag-nanoparticles is well established, although they face certain shortcomings due to toxicity to mammalian cells and limited penetration in biofilm matrices [153]. Recent studies [154] have been focused on countering these issues for example by developing Ag ring-coated super-paramagnetic IONPs (SPIONS) with ligand gaps. This has demonstrated high antimicrobial activity and remarkable compatibility with healthy host cells, which further exhibited enhanced activity against biofilm infections due to deeper penetration under an external magnetic field [155]. Others inorganic nanomaterials such as Au, Cu, Ni, Ti, Zn, graphene-based photo-thermal, as well as their coupled derivatives, are potential candidate for enhancing or restoring the already existing antibiotics or new substances to combat the multi-drug-resistance (MDR) problem (Figure 1).

The nanoparticles are indeed potential broad spectrum antibiotics because they can inhibit a wide range of MDR bacteria which have defied most of antibiotic treatments [152]. For example, CuO-nanoparticles exert their antibacterial activity by membrane disruption and ROS production [156], showing an antibacterial efficacy alike to Ag or ZnO. On the other hand, ZnO-nanoparticles, which are more effective, affect bacterial cell along two pathways: (1) by binding to membranes, disrupting their potential and integrity, and (2) by inducting ROS production [151]. Hence, ZnO-nanoparticles inhibit the growth of MSSA, MRSA, MDR or pathogens such as Streptococcus mutans, Lactobacillus, Klebsiella pneumoniae or E. coli [151], including ESBL producers [157], but also prevent biofilm formation [158] (Figure 1).

Nanoshuttle systems deliver antibiotics to a precise location and release them progressively in a controlled manner (shuttle systems). These systems use nanoparticles for the delivery and controlled released of several antibiotics and natural products. Nanoparticles are free to move uninhibited into
cells, increasing their efficiency. Antibiotics can be released inside the microorganism, increasing the therapeutic index and reducing the overall serum concentration. As a result, the deleterious side effects decrease [159]. An additional advantage is the decrease risk of creating resistance in other commensal microorganisms [160]. Most applications are focusing on the treatment of osteomyelitis [161], skin or wound [162] or *S. aureus*, *E. coli* or *Pseudomonas aeruginosa* infections [163]. The antimicrobial nanomaterials currently in use or under investigation are based on Ag, magnetite, TiO$_2$ and ZnO. Nanotechnology is also making great progress in combating infections associated with medical devices (such as those related with biofilms formation), with the implementation of tailored coating systems. These systems are based on coating the surface with nanoparticles inhibiting biofilm formation. Most studies have been focused on pathogens frequently associated with nosocomial infections such as *S. aureus*, *P. aeruginosa*, *Acinetobacter baumannii* and *K. pneumoniae* [160] (Figure 1). Min et al. [164] have demonstrated the applicability of coated degradable multilayer prosthesis. These coated prosthesis sequentially deliver the antibiotic and the osteo-inductive growth factor (BMP-2). This coating delivery system enables both eradication of established biofilms, as well as, a complete and rapid bone tissue repair around the implant in rats with induced osteomyelitis [164]. Their findings demonstrated the potential of this layered release strategy. Milo et al. [165] published a novel and previously unreported application of a pH-responsive polymer, in a dual-layered surface coating for urinary catheters that provides a visual early warning of *Proteus mirabilis* infection and their subsequent blockage control.

Nanoparticles keep offering promising alternatives in the design of effective next-generation therapeutics against bacterial, viral and fungal threats [155]. The perspective of developing powerful nano-antimicrobial agents with multiple-functionality will revolutionize clinical medicine and it will play a significant role in alleviating disease burden [152]. Nanoparticle-based antimicrobial agents can be used in ex vivo applications such as sterilizers for surfaces and devices, and the prospective topical applications for wound healing of nanoparticles-based systems [155] (Figure 1).

Currently, some drug delivery systems (DDS) usually named “nano-antibiotics” have been clinically-approved for human use in various infectious diseases, among them, liposomal delivery systems. Pulmaquin™ and Lipoquin™ (Grifols, S.A., Barcelona, Spain and Aradigm Corporation, Hayward, CA, USA) are inhalable liposomal dosage forms of ciprofloxacin, for the treatment of serious infectious diseases encountered in cystic fibrosis (CF) or in non-CF bronchiectasis. AX-Tobra™ (Axentis Pharma, Zurich, Switzerland) based on Fluidosomes® technology and Arikace® (Insmed Inc., Monmouth Junction, NJ, USA) undergoing phase III clinical trials, are respectively, an inhalable liposomal tobramycin and amikacin dosage forms, claimed for the treatment of *P. aeruginosa* pulmonary infections in cf. [166].

### 5. Conclusions

Nanobiotechnology offers multiple solutions for the prevention, diagnosis and treatment of infectious diseases. Nanoparticles can be designed to increase the activity in vaccines with low toxicity against the host. A huge number of nanoparticles can be used for the delivery and stabilization of bioactive drugs as well as antimicrobial molecules, ensuring controlled release of the drug. Due to their specificity, low dimensions, targeted delivery, controlled release properties and low cytotoxicity, nano-active systems could lead to more efficient and less invasive therapeutic outcome, contributing to the development of personalized treatment for several infectious diseases.

In the superbug era, nanotechnology is offering a new approach that allows us to fight against resistant bacteria. Nanobiomedicine offers new tools to be applied in the prevention, detection and treatment of the infectious diseases, managing to decrease the co-morbidity/mortality ratios, costs and improving lifestyle quality.

**Acknowledgments:** We acknowledge the helpful support and advice from Prof. Carlos García Riestra (Lab of Microbiology, University Hospital Complex of Santiago de Compostela and Department of Microbiology at USC) and Eric Harvill Department of Infectious Diseases; College of Veterinary Medicine, University of Georgia, Athens,
USA. This work has benefited by the financial support of a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS–UEFISCDI, project number PN-II-RU-TE-2014-4-2269.

**Author Contributions:** M.C.G., A.M.H. and E.T.-S. have written the review.

**Conflicts of Interest:** The authors declare no competing financial interest.

**Abbreviations**

- ACP: Antigen Presenting Cells
- Ag: Antigen
- BT: Botulinum toxin
- CF: Cystic fibrosis
- CFU: Colony Forming Unit
- ELISA: Enzyme Linked Immunosorbent Assay
- FSNP: Fluorescent Silica Nanoparticles
- HBV: Hepatitis B Virus
- HCV: Hepatitis C Virus
- HIV: Human Immunodeficiency Virus
- Ig: Immunoglobulin
- IONP: Iron oxide nanoparticles
- MALDI-TOF MS: Matrix Assisted Laser Desorption Ionization—Time of Flight Mass Spectrometry
- MNP: Magnetic nanoparticles
- QD: Quantum dots
- ROS: Reactive Oxygen Species
- SiNP: Silica-based nanoparticles
- VLP: Virus-like particles
- WHO: World Health Organization

**References**

1. Feynman, R.P. Plenty of room at the bottom. *Am. Phy. Soc.* 1959. Available online: http://www.pa.msu.edu/~yang/RFeynman PlentySpace.pdf (accessed on 30 June 2016).
2. Plenty of room’ revisited. *Nat. Nanotech.* 2009, 4, 781. [CrossRef]
3. Drexler, K.E. Molecular engineering: An approach to the development of general capabilities for molecular manipulation. *Proc. Natl. Acad. Sci. USA* 1981, 78, 5275–5278. [CrossRef] [PubMed]
4. Drexler, K.E. *Engines of Creation: The Coming Era of Nanotechnology*; Doubleday: New York City, NY, USA, 1986.
5. Savage, N.; Thomas, T.A.; Duncan, J.S. Nanotechnology applications and implications research supported by the US environmental protection agency star grants program. *J. Environ. Monit.* 2007, 9, 1046–1054. [CrossRef] [PubMed]
6. Medina, C.; Santos-Martinez, M.J.; Radomski, A.; Corrigan, O.I.; Radomski, M.W. Nanoparticles: Pharmacological and toxicological significance. *Br. J. Pharmacol.* 2007, 150, 552–558. [CrossRef] [PubMed]
7. Jos, A.; Pichardo, S.; Puerto, M.; Sanchez, E.; Grilo, A.; Camean, A.M. Cytotoxicity of carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line caco-2. *Toxicol. In Vitro* 2009, 23, 1491–1496. [CrossRef] [PubMed]
8. Holban, A.M.; Gestal, M.C.; Grumezescu, A.M. Control of biofilm-associated infections by signaling molecules and nanoparticles. *Int. J. Pharm.* 2016, in press. [CrossRef] [PubMed]
9. Holban, A.M.; Gestal, M.C.; Grumezescu, A.M. New molecular strategies for reducing implantable medical devices associated infections. *Current Med. Chem.* 2014, 21, 3375–3382. [CrossRef]
10. Lara, H.H.; Romero-Urbina, D.G.; Pierce, C.; Lopez-Ribot, J.L.; Arellano-Jimenez, M.J.; Jose-Yacaman, M. Effect of silver nanoparticles on candida albicans biofilms: An ultrastructural study. *J. Nanobiotechnol.* 2015, 13, 91. [CrossRef] [PubMed]
11. Veigas, B.; Fernandes, A.R.; Baptista, P.V. Aunps for identification of molecular signatures of resistance. *Front. Microbiol.* 2014, 5, 455. [CrossRef] [PubMed]
12. Mirkin, C.A.; Letsinger, R.L.; Mucic, R.C.; Storhoff, J.J. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* 1996, 382, 607–609. [CrossRef] [PubMed]
13. Bailey, R.C.; Nam, J.M.; Mirkin, C.A.; Hupp, J.T. Real-time multicolor DNA detection with chemoresponsive diffraction gratings and nanoparticle probes. *J. Am. Chem. Soc.* 2003, 125, 13541–13547. [CrossRef] [PubMed]
14. Salvador, A.; Igartua, M.; Hernandez, R.M.; Pedraz, J.L. An overview on the field of micro- and nanotechnologies for synthetic peptide-based vaccines. J. Drug Deliv. 2011, 2011, 181646. [CrossRef] [PubMed]
15. Angsantikul, P.; Thamphiwatana, S.; Gao, W.; Zhang, L. Cell membrane-coated nanoparticles as an emerging antibacterial vaccine platform. Vaccines 2015, 3, 814–828. [CrossRef] [PubMed]
16. Fahmy, T.M.; Demento, S.L.; Caplan, M.J.; Mellman, I.; Saltzman, W.M. Design opportunities for actively targeted nanoparticle vaccines. Nanomedicine 2008, 3, 343–355. [CrossRef] [PubMed]
17. Irvine, D.J.; Hanson, M.C.; Rakha, K.; Tokatlian, T. Synthetic nanoparticles for vaccines and immunotherapy. Chem. Rev. 2015, 115, 11109–11146. [CrossRef] [PubMed]
18. Moghimi, S.M.; Hunter, A.C.; Murray, J.C. Nanomedicine: Current status and future prospects. FASEB J. 2005, 19, 311–330. [CrossRef] [PubMed]
19. Mateescu, A.L.; Dimov, T.V.; Grumezescu, A.M.; Gestal, M.C.; Chifiriuc, M.C. Nanostructured bioactive polymers used in food-packaging. Curr. Pharm. Biotechnol. 2015, 16, 121–127. [CrossRef] [PubMed]
20. Zhao, L.; Seth, A.; Wibowo, N.; Zhao, C.X.; Mitter, N.; Yu, C.; Middelberg, A.P. Nanoparticles and their coating: Role in cancer chemotherapy and drug delivery. Curr. Med. Chem. 2014, 21, 1051–1058. [CrossRef] [PubMed]
21. Kim, S.Y.; Doh, H.J.; Jang, M.H.; Ha, Y.J.; Chung, S.I.; Park, H.J. Oral immunization with helicobacter pylori-loaded poly(D,L-lactide-co-glycolide) nanoparticles. Helicobacter 1999, 4, 33–39. [CrossRef] [PubMed]
22. Saade, F.; Honda-Okubo, Y.; Trec, S.; Petrovsky, N. A novel hepatitis B vaccine containing adjuvant, induces robust humoral and cellular immune responses. J. Control. Release 2004, 17, 174–185. [CrossRef] [PubMed]
23. Parlane, N.A.; Rehm, B.H.; Wedlock, D.N.; Buddle, B.M. Novel particulate vaccines utilizing polyester nanoparticles (bio-beads) for protection against mycobacterium bovis infection—A review. Vet. Immunol. Immunopathol. 2014, 158, 8–13. [CrossRef] [PubMed]
24. Li, P.; Luo, Z.; Liu, P.; Gao, N.; Zhang, Y.; Pan, H.; Liu, L.; Wang, C.; Cai, L.; Ma, Y. Bioreducible alginat-poly(ethyleneimine) nanogels as an antigen-delivery system robustly enhance vaccine-elicited humoral and cellular immune responses. J. Control. Release 2013, 168, 271–279. [CrossRef] [PubMed]
25. Saade, F.; Honda-Okubo, Y.; Trec, S.; Petrovsky, N. A novel hepatitis B vaccine containing adjuvant, induces robust humoral and cellular immunity with minimal reactivity in preclinical testing. Vaccine 2013, 31, 1999–2007. [CrossRef] [PubMed]
26. Feng, G.; Jiang, Q.; Xia, M.; Lu, Y.; Qiu, W.; Zhao, D.; Lu, L.; Peng, G.; Wang, Y. Enhanced immune response and protective effects of nano-chitosan-based DNA vaccine encoding T cell epitopes of esat-6 and FL against mycobacterium tuberculosis infection. PLoS ONE 2013, 8, e61135. [CrossRef] [PubMed]
27. Tahara, Y.; Mukai, S.A.; Sawada, S.; Sasaki, Y.; Akiyoshi, K. Nanocarrier-integrated microspheres: Nanogel tectonic engineering for advanced drug-delivery systems. Adv. Mater. 2015, 27, 5080–5088. [CrossRef] [PubMed]
28. Li, S.; Rizzo, M.A.; Bhattacharya, S.; Huang, L. Characterization of cationic lipid-protamine-DNA (IPD) complexes for intravenous gene delivery. Gene Ther. 1998, 5, 930–937. [CrossRef] [PubMed]
29. Morein, B.; Lovgren, K.; Hoglund, S.; Sundquist, B. The iscom: An immunostimulating complex. Immunol. Today 1987, 8, 333–338. [CrossRef] [PubMed]
30. Zhang, L.F.; Zhou, J.; Chen, S.; Cai, L.L.; Bao, Q.Y.; Zheng, F.Y.; Lu, J.Q.; Padmanabha, J.; Hengst, K.; Malcolm, K.; et al. Protective effects of nano-chitosan-based DNA vaccine encoding T cell epitopes of esat-6 and FL against mycobacterium tuberculosis infection. PLoS ONE 2013, 8, e61135. [CrossRef] [PubMed]
31. Andre, F.E. Overview of a 5-year clinical experience with a yeast-derived hepatitis B vaccine. Vaccine 2015, 33, 5080–5088. [CrossRef] [PubMed]
32. Lopez-Sagaseta, J.; Malito, E.; Rappuoli, R.; Bottomley, M.J. Self-assembling protein nanoparticles in the design of vaccines. Comput. Struct. Biotechnol. J. 2016, 14, 58–68. [CrossRef] [PubMed]
33. Xu, Y.; Zhang, N.Z.; Wang, M.; Dong, H.; Feng, S.Y.; Guo, H.C.; Zhu, X.Q. A long-lasting protective immunity against chronic toxoplasmosis in mice induced by recombinant rhophry proteins encapsulated in poly (lactide-co-glycolide) microparticles. Parasitol. Res. 2015, 114, 4195–4203. [CrossRef] [PubMed]
34. Mohan, T.; Mitra, D.; Rao, D.N. Nasal delivery of plg microparticle encapsulated defensin peptides adjuvanted GP41 antigen confers strong and long-lasting immunoprotective response against HIV-1. Immunol. Res. 2014, 58, 139–153. [CrossRef] [PubMed]
35. Colonna, C.; Dorati, R.; Conti, B.; Caliceti, P.; Genta, I. Sub-unit vaccine against S. aureus-mediated infections: Set-up of nano-sized polymeric adjuvant. Int. J. Pharm. 2013, 452, 390–401. [CrossRef] [PubMed]
36. Lawlor, C.; O’Connor, G.; O’Leary, S.; Gallagher, P.J.; Cryan, S.A.; Keane, J.; O’Sullivan, M.P. Treatment of mycobacterium tuberculosis-infected macrophages with poly(lactic-co-glycolic acid) microparticles drives \( \text{nrf2} \) and autophagy dependent bacillary killing. *PLoS ONE* **2016**, *11*, e0149167. [CrossRef] [PubMed]

37. Singh, D.; Somani, V.K.; Morasse, A.; Evans, J.T.; Burkhart, D.J. Peg modified liposomes containing CRX-601 adjuvant in combination with methylglycol chitosan enhance the murine sublingual immune response to influenza vaccination. *J. Control. Release* **2016**, *223*, 64–74. [CrossRef] [PubMed]

38. Liu, Y.; Balachandran, Y.L.; Li, D.; Shao, Y.; Jiang, X. Polyvinylpyrrolidone-poly(ethylene glycol) modified silver nanorods can be a safe, noncarrier adjuvant for HIV vaccine. *ACS Nano* **2016**, *10*, 3589–3596. [CrossRef] [PubMed]

39. Oberoi, H.S.; Yorgensen, Y.M.; Morasse, A.; Evans, J.T.; Burkhart, D.J. Peg modified liposomes containing CRX-601 adjuvant in combination with methylglycol chitosan enhance the murine sublingual immune response to influenza vaccination. *J. Control. Release* **2016**, *223*, 64–74. [CrossRef] [PubMed]

40. Powles, L.; Xiang, S.D.; Selomulya, C.; Plebanski, M. The use of synthetic carriers in malaria vaccine design. *Vaccines* **2015**, *3*, 894–929. [CrossRef] [PubMed]

41. Parlane, N.A.; Grage, K.; Mifune, J.; Basaraba, R.J.; Wedlock, D.N.; Rehm, B.H.; Buddle, B.M. Vaccines displaying mycobacterial proteins on biopolyester beads stimulate cellular immunity and induce protection against tuberculosis. *Clin. Vaccine Immunol.* **2012**, *19*, 37–44. [CrossRef] [PubMed]

42. Feinen, B.; Petrovsky, N.; Verma, A.; Merkel, T.J. Advax-adjuvant recombinant protective antigen provides protection against inhalational anthrax that is further enhanced by addition of muramabutide adjuvant. *Clin. Vaccine Immunol.* **2014**, *21*, 580–586. [CrossRef] [PubMed]

43. Rodriguez-Del Rio, E.; Marradi, M.; Calderon-Gonzalez, R.; Frande-Cabanes, E.; Penades, S.; Petrovsky, N.; Alvarez-Dominguez, C. A gold glyco-nanoparticle carrying a listeriolysin o peptide and formulated with advax delta inulin adjuvant induces robust T-cell protection against listeria infection. *Vaccine* **2015**, *33*, 1465–1473. [CrossRef] [PubMed]

44. Honda-Okubo, Y.; Saade, F.; Petrovsky, N. Advax, a polysaccharide adjuvant derived from delta inulin, provides improved influenza vaccine protection through broad-based enhancement of adaptive immune responses. *Vaccine* **2012**, *30*, 5373–5381. [CrossRef] [PubMed]

45. Honda-Okubo, Y.; Barnard, D.; Ong, C.H.; Peng, B.H.; Tseng, C.T.; Petrovsky, N. Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. *J. Virol.* **2015**, *89*, 2995–3007. [CrossRef] [PubMed]

46. Petrovsky, N.; Cooper, P.D. Advax, a novel microcrystalline polysaccharide particle engineered from delta inulin, provides robust adjuvant potency together with tolerability and safety. *Vaccine* **2015**, *33*, 5920–5926. [CrossRef] [PubMed]

47. Jain, R.R.; Mehta, M.R.; Bannalikar, A.R.; Menon, M.D. Alginate microparticles loaded with lipopolysaccharide subunit antigen for mucosal vaccination against *Klebsiella pneumoniae*. *Biologicals* **2015**, *33*, 195–201. [CrossRef] [PubMed]

48. Farjah, A.; Owlia, P.; Siadat, S.D.; Mousavi, S.F.; Ardestani, M.S.; Mohammadpour, H.K. Immunological evaluation of an alginate-based conjugate as a vaccine candidate against *Pseudomonas aeruginosa*. *Acta Pathol. Microbiol. Immunol. Scand.* **2015**, *123*, 175–183. [CrossRef] [PubMed]

49. Nagatomo, D.; Tanai, M.; Ariyasu, H.; Taniguchi, M.; Aga, M.; Ariyasu, T.; Ohta, T.; Fukuda, S. Cholesteryl pullulan-encapsulated TNF-alpha nanoparticles are an effective mucosal vaccine adjuvant against influenza virus. *Biomed. Res. Int.* **2015**, *2015*, 471468. [CrossRef] [PubMed]

50. Cevher, E.; Salomon, S.K.; Somavarapu, S.; Brocchini, S.; Alpar, H.O. Development of chitosan-pullulan composite nanoparticles for nasal delivery of vaccines: In vivo studies. *J. Microencapsul.* **2015**, *32*, 769–783. [CrossRef] [PubMed]

51. Doavi, T.; Mousavi, S.L.; Kamali, M.; Amani, J.; Fasihi Ramandi, M. Chitosan-based intranasal vaccine against *Escherichia coli* O157:H7. *Iran Biomed.* J. **2016**, *20*, 97–108. [PubMed]

52. Cui, Z.; Han, D.; Sun, X.; Zhang, M.; Feng, X.; Sun, C.; Gu, J.; Tong, C.; Lei, L.; Han, W. Mannose-modified chitosan microspheres enhance oprf-opri-mediated protection of mice against *Pseudomonas aeruginosa* infection via induction of mucosal immunity. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 667–680. [CrossRef] [PubMed]
53. Khalili, I.; Ghadimipour, R.; Sadigh Eteghad, S.; Fathi Najafi, M.; Ebrahimi, M.M.; Godsian, N.; Seﬁdi Heris, Y; Khalili, M.T. Evaluation of immune response against inactivated avian inﬂuenza (H9N2) vaccine, by using chitosan nanoparticles. *Jundishapur. J. Microbiol.* 2015, 8, e27035. [CrossRef] [PubMed]

54. Lebre, F.; Borchard, G.; Faneca, H.; Pedroso de Lima, M.C.; Borges, O. Intranasal administration of novel chitosan nanoparticle/DNA complexes induces antibody response to hepatitis B surface antigen in mice. *Mol. Pharm.* 2016, 13, 472–482. [CrossRef] [PubMed]

55. Malathi, B.; Mona, S.; Thiayagarajan, D.; Kaliraj, P. Immunopotentiating nano-chitosan as potent vaccine carrier for efﬁcacious prophylaxis of ﬁlarial antigens. *Int. J. Biol. Macromol.* 2015, 73, 131–137. [CrossRef] [PubMed]

56. Hunsawong, T.; Sunintaboon, P.; Warit, S.; Thaisomboonsuk, B.; Jarman, R.G.; Yoon, I.K.; Ubol, S.; Fernandez, S. A novel dengue virus serotype-2 nanovaccine induces robust humoral and cell-mediated immunity in mice. *Vaccine* 2015, 33, 1702–1710. [CrossRef] [PubMed]

57. Fukuyama, Y.; Yuki, Y.; Katakai, Y.; Harada, N.; Takahashi, H.; Takeda, S.; Mejima, M.; Joo, S.; Kurokawa, S.; Sawada, S.; et al. Nanogel-based pneumococcal surface protein a nasal vaccine induces microRNA-associated TH17 cell responses with neutralizing antibodies against streptococcus pneumoniae in macaques. *Mucosal Immunol.* 2015, 8, 1144–1153. [CrossRef] [PubMed]

58. Xu, J.H.; Dai, W.J.; Chen, B.; Fan, X.Y. Mucosal immunization with psaa protein, using chitosan as a delivery system, increases protection against acute otitis media and invasive infection by streptococcus pneumoniae. *Scand. J. Immunol.* 2015, 81, 177–185. [CrossRef] [PubMed]

59. Tahamtan, A.; Ghaemi, A.; Gorji, A.; Kalhor, H.R.; Sajadian, A.; Tabarraei, A.; Moradi, A.; Atyabi, F.; Kelishadi, M. Antitumor effect of therapeutic hpv DNA vaccines with chitosan-based nanodelivery systems. *J. Biomed. Sci.* 2014, 21, 69. [CrossRef] [PubMed]

60. Dai, C.; Kang, H.; Yang, W.; Sun, J.; Liu, C.; Cheng, G.; Rong, G.; Wang, X.; Wang, X.; Jin, Z.; et al. O-2’-hydroxypropyltrimethyl ammonium chloride chitosan nanoparticles for the delivery of live newcastle disease vaccine. *Carbohydr. Polym.* 2015, 130, 280–289. [CrossRef] [PubMed]

61. Hunsawong, T.; Sunintaboon, P.; Warit, S.; Thaisomboonsuk, B.; Jarman, R.G.; Yoon, I.K.; Ubol, S.; Fernandez, S. Immunogenic properties of a bcg adjuvanted chitosan nanoparticle-based dengue vaccine in human dendritic cells. *PLoS Negl. Trop. Dis.* 2015, 9, e0003958. [CrossRef] [PubMed]

62. Boraschi, D.; Italiani, P. From antigen delivery system to adjuvancity: The board application of nanoparticles in vaccinology. *Vaccines* 2015, 3, 930–939. [CrossRef] [PubMed]

63. Qiao, C.; Liu, J.; Yang, J.; Li, Y.; Weng, J.; Shao, Y.; Zhang, X. Enhanced non-inflammasome mediated immune responses by mannosylated zwitterionic-based cationic liposomes for HIV DNA vaccines. *Biomaterials* 2016, 85, 1–17. [CrossRef] [PubMed]

64. Monaci, E.; Mancini, F.; Lofano, G.; Bacconi, M.; Tavarini, S.; Sammicheli, C.; Arcidiacono, L.; Giraldi, M.; Galletti, B.; Rossi Paccani, S.; et al. Mf59- and al(OH)3-adjuvanted staphylococcus aureus (4C-Staph) vaccines induce sustained protective humoral and cellular immune responses, with a critical role for effector CD4 T cells at low antibody titers. *Front. Immunol.* 2015, 6, 439. [CrossRef] [PubMed]

65. Klimka, A.; Michels, L.; Glowalla, E.; Tosetti, B.; Kronke, M.; Krut, O. Montanide isa 71 VG is advantageous to freund’s adjuvant in immunization against *S. aureus* infection of mice. *Scand. J. Immunol.* 2015, 81, 291–297. [CrossRef] [PubMed]

66. Wilson, K.L.; Xiang, S.D.; Plebanski, M. A model to study the impact of polymorphism driven liver-stage immune evasion by malaria parasites, to help design effective cross-reactive vaccines. *Front. Microbiol.* 2016, 7, 303. [CrossRef] [PubMed]

67. Cargnelutti, D.E.; Salomon, M.C.; Celedon, V.; Garcia Bustos, M.F.; Morea, G.; Cuello-Carrion, F.D.; Scodeller, E.A. Immunization with antigenic extracts of *Leishmania* associated with Montanide ISA 763 adjuvant induces partial protection in BALB/c mice against *Leishmania (Leishmania) amazonensis* infection. *J. Microbiol. Immunol. Infect.* 2016, 49, 24–32. [CrossRef] [PubMed]

68. Safari, D.; Marradi, M.; Chiodo, F.; Th Dekker, H.A.; Shan, Y.; Adamo, R.; Oscarson, S.; Rijkers, G.T.; Lahmann, M.; Kamerling, J.P.; et al. Gold nanoparticles as carriers for a synthetic streptococcus pneumoniae type 14 conjugate vaccine. *Nanomedicine* 2012, 7, 651–662. [CrossRef] [PubMed]

69. Gregory, A.E.; Judy, B.M.; Qazi, O.; Blumentritt, C.A.; Brown, K.A.; Shaw, A.M.; Torres, A.G.; Titball, R.W. A gold nanoparticle-linked glycoconjugate vaccine against burkholderia mallei. *Nanomedicine* 2015, 11, 447–456. [CrossRef] [PubMed]
70. Gregory, A.E.; Williamson, E.D.; Prior, J.L.; Butcher, W.A.; Thompson, I.J.; Shaw, A.M.; Titball, R.W. Conjugation of Y. pestis F1-antigen to gold nanoparticles improves immunogenicity. *Vaccine* 2012, 30, 6777–6782. [CrossRef] [PubMed]

71. Kumar, R.; Ray, P.C.; Datta, D.; Bansal, G.P.; Angov, E.; Kumar, N. Nanovaccines for malaria using *Plasmodium falciparum* antigen Pf25 attached gold nanoparticles. *Vaccine* 2015, 33, 5064–5071. [CrossRef] [PubMed]

72. Gianvincenzo, P.D.; Calvo, J.; Perez, S.; Alvarez, A.; Bedoya, L.M.; Alcami, J.; Penades, S. Negatively charged glyconanoparticles modulate and stabilize the secondary structures of a gp120 V3 loop peptide: Toward fully synthetic HIV vaccine candidates. *Bioconj. Chem.* 2015, 26, 755–765. [CrossRef] [PubMed]

73. Yandar, N.; Pastorin, G.; Prato, M.; Bianco, A.; Patarroyo, M.E.; Lozano, J.M. Immunological profile of a *Plasmodium vivax* AMA-1 N-terminus peptide-carbon nanotube conjugate in an infected plasmodium berghei mouse model. *Vaccine* 2008, 26, 5864–5873. [CrossRef] [PubMed]

74. Andrade, G.R.; New, R.R.; Sant’Anna, O.A.; Williams, N.A.; Alves, R.C.; Pimenta, D.C.; Vigerelli, H.; Melo, B.S.; Rocha, L.B.; Piazza, R.M.; et al. A universal polysaccharide conjugated vaccine against O111 *E. coli*. *Hum. Vaccines Immunother.* 2014, 10, 2864–2874. [CrossRef] [PubMed]

75. Neuhaus, V.; Chichester, J.A.; Ebensen, T.; Schwarz, K.; Hartman, C.E.; Shoji, Y.; Guzman, C.A.; Yusibov, V.; Sewald, K.; Braun, A. A new adjuvanted nanoparticle-based H1N1 influenza vaccine induced antigen-specific local mucosal and systemic immune responses after administration into the lung. *Vaccine* 2014, 32, 3216–3222. [CrossRef] [PubMed]

76. Skrastina, D.; Petrovskis, I.; Lieknina, I.; Bogans, J.; Renhofa, R.; Ose, V.; Dishlers, A.; Dekhtyar, Y.; Pumpens, P. Silica nanoparticles as the adjuvant for the immunisation of mice using hepatitis B core virus-like particles. *PLoS ONE* 2014, 9, e114006. [CrossRef] [PubMed]

77. Saeed, M.I.; Omar, A.R.; Hussein, M.Z.; Elkhidir, I.M.; Sekawi, Z. Systemic antibody response to nano-size calcium phosphate biocompatible adjuvant adsorbed HEV-71 killed vaccine. *Clin. Exp. Vaccine Res.* 2015, 4, 88–98. [CrossRef] [PubMed]

78. Viswanathan, K.; Gopinath, V.P.; Raj, G.D. Formulation of newcastle disease virus coupled calcium phosphate nanoparticles: An effective strategy for oculonasal delivery to chicken. *Colloids Surf. B Biointerfaces* 2014, 116, 9–16. [CrossRef] [PubMed]

79. Knuschke, T.; Bayer, W.; Rotan, O.; Sokolova, V.; Wadwa, M.; Kirschning, C.J.; Hansen, W.; Dittmer, U.; Epple, M.; Buer, J.; et al. Prophylactic and therapeutic vaccination with a nanoparticle-based peptide vaccine induces efficient protective immunity during acute and chronic retroviral infection. *Nanomedicine* 2014, 10, 1787–1798. [CrossRef] [PubMed]

80. Renna, M.S.; Pereyra, E.A.; Baravalle, C.; Camussone, C.M.; Dallard, B.E.; Marcipar, I.S.; Calvinho, L.F. Functional role of antibodies generated in heifers through immunization with staphylococcus aureus vaccines in invasion and phagocytosis assays. *FEMS Microbiol. Lett.* 2014, 360, 62–69. [CrossRef] [PubMed]

81. Bontempi, I.A.; Vicco, M.H.; Cabrera, G.; Villar, S.R.; Gonzalez, F.B.; Roggero, E.A.; Alcami, J.; Penades, S. Efficacy of a trans-sialidase iscom-matrix subunit vaccine candidate to protect against experimental chagas disease. *Vaccine* 2015, 33, 1274–1283. [CrossRef] [PubMed]

82. Heldens, J.G.; Pouwels, H.G.; Derks, C.G.; Van de Zande, S.M.; Hoeijmakers, M.J. Duration of immunity induced by an equine influenza and tetanus combination vaccine formulation adjuvanted with iscom-matrix. *Vaccine* 2016, 34, 1589–1596. [CrossRef] [PubMed]

83. Lee, K.L.; Twyman, R.M.; Fiering, S.; Steinmetz, N.F. Virus-based nanoparticles as platform technologies for modern vaccines. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 2016, 8, 554–578. [CrossRef] [PubMed]

84. Zhu, R.; Liu, J.; Chen, C.; Ye, X.; Xu, L.; Wang, W.; Zhao, Q.; Zhu, H.; Cheng, T.; Xia, N. A highly conserved epitope-vaccine candidate against varicella-zoster virus induces neutralizing antibodies in mice. *Vaccine* 2016, 34, 1589–1596. [CrossRef] [PubMed]

85. Ghasemi, F.; Rostami, S.; Meshkat, Z. Progress in the development of vaccines for hepatitis c virus infection. *World J. Gastroenterol.* 2015, 21, 11984–12002. [CrossRef] [PubMed]

86. Yee, P.T.; Poh, C.L. Development of novel vaccines against enterovirus-71. *Viruses* 2015, 8. [CrossRef] [PubMed]

87. Schwameis, M.; Buchtele, N.; Wadowski, P.P.; Schoerenghofer, C.; Jilma, B. Chikungunya vaccines in development. *Hum. Vaccines Immunother.* 2016, 12, 716–731. [CrossRef] [PubMed]
88. Tamborrini, M.; Geib, N.; Marrero-Nodarse, A.; Jud, M.; Hauser, J.; Aho, C.; Lamelas, A.; Zuniga, A.; Pluschke, G.; Ghasparian, A.; et al. A synthetic virus-like particle streptococcal vaccine candidate using B-cell epitopes from the proline-rich region of pneumococcal surface protein A. *Vaccines* 2015, 3, 850–874. [CrossRef] [PubMed]

89. McKay, P.F.; Cope, A.V.; Mann, J.F.; Joseph, S.; Esteban, M.; Tatoud, R.; Carter, D.; Reed, S.G.; Weber, J.; Shattock, R.J. Glucopyranosyl lipid a adjuvant significantly enhances HIV specific T and B cell responses elicited by a DNA-mva-protein vaccine regimen. *PLoS ONE* 2014, 9, e84707. [CrossRef] [PubMed]

90. Burkhard, P.; Lanar, D.E. Malaria vaccine based on self-assembling protein nanoparticles. *Expert Rev. Vaccines* 2015, 14, 1525–1527. [CrossRef] [PubMed]

91. El Bissati, K.; Zhou, Y.; Dasgupta, D.; Cobb, D.; Dubey, J.P.; Burkhard, P.; Lanar, D.E.; McLeod, R. Effectiveness of a novel immunogenic nanoparticle platform for toxoplasma peptide vaccine in HLA transgenic mice. *Vaccine* 2014, 32, 3243–3248. [CrossRef] [PubMed]

92. Babapoor, S.; Neef, T.; Mittelholzer, C.; Girshick, T.; Garmendia, A.; Shang, H.; Khan, M.I.; Burkhard, P. A novel vaccine using nanoparticle platform to present immunogenic M2e against avian influenza infection. *Influenza Res. Treat.* 2011, 2011, 126794. [CrossRef] [PubMed]

93. Wahome, N.; Pfeiffer, T.; Ambiel, I.; Yang, Y.; Keppler, O.T.; Bosch, V.; Burkhard, P. Conformation-specific display of 4E10 and 2F5 epitopes on self-assembling protein nanoparticles as a potential HIV vaccine. *Chem. Biol. Drug Des.* 2012, 80, 349–357. [CrossRef] [PubMed]

94. Qasim, M.; Lim, D.J.; Park, H.; Na, D. Nanotechnology for diagnosis and treatment of infectious diseases. *J. Nanosci. Nanotechnol.* 2014, 14, 7374–7387. [CrossRef] [PubMed]

95. Pedrosa, P.; Baptista, P.V. Gold and silver nanoparticles for diagnostics of infection. In *Nanotechnology in Diagnosis, Treatment and Prophylaxis of Infectious Diseases*, 1st ed; Elsevier: London, UK, 2015; Chapter: 1. [CrossRef]

96. Hauck, T.S.; Giri, S.; Gao, Y.; Chan, W.C. Nanotechnology diagnostics for infectious diseases prevalent in developing countries. *Adv. Drug Deliv. Rev.* 2010, 62, 438–448. [CrossRef] [PubMed]

97. Blecher, K.; Nasir, A.; Friedman, A. The growing role of nanotechnology in combating infectious disease. *Virulence* 2011, 2, 395–401. [CrossRef] [PubMed]

98. Qin, D.; He, X.; Wang, K.; Zhao, X.J.; Tan, W.; Chen, J. Fluorescent nanoparticle-based indirect immunofluorescence microscopy for detection of mycobacterium tuberculosis. *J. Biomed. Biotechnol.* 2007, 2007, 89364. [CrossRef] [PubMed]

99. Shangguan, J.; Li, Y.; He, D.; He, X.; Wang, K.; Zou, Z.; Shi, H. A combination of positive dielectrophoresis driven on-line enrichment and aptamer-fluorescent silica nanoparticle label for rapid and sensitive detection of staphylococcus aureus. *Analyst* 2015, 140, 4489–4497. [CrossRef] [PubMed]

100. Wang, Q.Y.; Kang, Y.J. Bioprobes based on aptamer and silica fluorescent nanoparticles for bacteria salmonella typhimurium detection. *Nanoscale Res. Lett.* 2016, 11, 150. [CrossRef] [PubMed]

101. Singh, A.K.; Harrison, S.H.; Schoeniger, J.S. Gangliosides as receptors for biological toxins: Development of sensitive fluor immunooassays using ganglioside-bearing liposomes. *Anal. Chem.* 2000, 72, 6019–6024. [CrossRef] [PubMed]

102. Ahn-Yoon, S.; DeCory, T.R.; Baeumner, A.J.; Durst, R.A. Ganglioside-liposome immunoassay for the ultrasensitive detection of cholera toxin. *Anal. Chem.* 2003, 75, 2256–2261. [CrossRef] [PubMed]

103. Ahn-Yoon, S.; DeCory, T.R.; Durst, R.A. Ganglioside-liposome immunoassay for the detection of botulinum toxin. *Anal. Bioanal. Chem.* 2004, 378, 68–75. [CrossRef] [PubMed]

104. De Mello Donega, C. Synthesis and properties of colloidal heteronanocrystals. *Chem. Soc. Rev.* 2011, 40, 1512–1546. [CrossRef] [PubMed]

105. Conde, J.; Dias, J.T.; Grazu, V.; Moros, M.; Baptista, P.V.; de la Fuente, J.M. Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine. *Front. Chem.* 2014, 2, 48. [CrossRef] [PubMed]

106. Medintz, I.L.; Mattoussi, H.; Clapp, A.R. Potential clinical applications of quantum dots. *Int. J. Nanomed.* 2008, 3, 151–167.

107. Zhang, C.Y.; Hu, J. Single quantum dot-based nanosensor for multiple DNA detection. *Anal. Chem.* 2010, 82, 1921–1927. [CrossRef] [PubMed]
Li, J.; Qin, T.; Jia, X.X.; Deng, A.H.; Zhang, X.; Fan, W.H.; Huo, S.D.; Wen, T.Y.; Liu, W.J. Rapid identification of pathogenic bacteria using biofunctionalized nanoparticles. *Molecules* 2016, 21, 867.

Ho, K.C.; Tsai, P.J.; Lin, Y.S.; Chen, Y.C. Using biofunctionalized nanoparticles to probe pathogenic bacteria. *Molecules* 2016, 21, 109–117.

Goldman, E.R.; Balighian, E.D.; Mattoussi, H.; Kuno, M.K.; Mauro, J.M.; Tran, P.T.; Anderson, G.P. Avidin: A natural bridge for quantum dot-antibody conjugates. *J. Am. Chem. Soc.* 2002, 124, 6378–6382.

Agrawal, A.; Tripp, R.A.; Anderson, L.J.; Nie, S. Real-time detection of virus particles and viral protein expression with two-color quantum dots. *J. Virol.* 2005, 79, 8625–8628.

Klostranec, J.M.; Xiang, Q.; Farcas, G.A.; Lee, J.A.; Rhee, A.; Lafferty, E.I.; Perrault, S.D.; Kain, K.C.; Chan, W.C. Convergence of quantum dot barcodes with microfluidics and signal processing for multiplexed high-throughput infectious disease diagnostics. *Nano Lett.* 2007, 7, 2812–2818.

Ebrahim, S.; Reda, M.; Hussien, A.; Zayed, D. CdTe quantum dots as a novel biosensor for *serratia marcescens* and lipopolysaccharide. *Acta A Mol. Biomol. Spectrosc.* 2015, 150, 212–219.

Goluch, E.D.; Nam, J.M.; Georganopoulou, D.G.; Chiesl, T.N.; Shaikh, K.A.; Ryu, K.S.; Barron, A.E.; Mirkin, C.A.; Liu, C. A bio-barcode assay for on-chip attomolar-sensitivity protein detection. *Lab Chip* 2006, 6, 1293–1299.

Duan, L.; Wang, Y.; Li, S.S.; Wan, Z.; Zhai, J. Rapid and simultaneous detection of human hepatitis B virus and hepatitis C virus antibodies based on a protein chip assay using nano-gold immunological amplification and silver staining method. *BMC Infect. Dis.* 2005, 5, 53.

Bohara, R.A.; Pawar, S.H. Innovative developments in bacterial detection with magnetic nanoparticles. *Appl. Biochem. Biotechnol.* 2015, 176, 1044–1058.

Yoo, J.W.; Doshi, N.; Mitragotri, S. Adaptive micro and nanoparticles: Temporal control over carrier properties to facilitate drug delivery. *Adv. Drug Deliv. Rev.* 2011, 63, 1247–1256.

Tsourkas, A.; Hofstetter, O.; Hofstetter, H.; Weissleder, R.; Josephson, L. Magnetic relaxation switch immunosensors detect enantiomeric impurities. *Angew. Chem.* 2004, 43, 2395–2399.

Ho, K.C.; Tsai, P.J.; Lin, Y.S.; Chen, Y.C. Using biofunctionalized nanoparticles to probe pathogenic bacteria. *Anal. Chem.* 2004, 76, 7162–7168.

Lin, Y.S.; Tsai, P.J.; Weng, M.F.; Chen, Y.C. Affinity capture using vancomycin-bound magnetic nanoparticles for the maldi-ms analysis of bacteria. *Anal. Chem.* 2005, 77, 1753–1760.

Gopal, J.; Muthu, M.; Chun, S.C.; Wu, H.F. State-of-the-art nanoplatform-integrated MALDI-MS impacting resolutions in urinary proteomics. *Proteom. Clin. Appl.* 2015, 9, 469–481.

Azzazy, H.M.; Mansour, M.M.; Kazmierczak, S.C. Nanodiagnostics: A new frontier for clinical laboratory medicine. *Clin. Chem.* 2006, 52, 1238–1246.

Chen, L.; Mungroo, N.; Daikwura, L.; Neethirajan, S. Label-free nir-sers discrimination and detection of foodborne bacteria by in situ synthesis of Ag colloids. *J. Nanobiotechnol.* 2015, 13, 45.

Li, J.; Qin, T.; Jia, X.X.; Deng, A.H.; Zhang, X.; Fan, W.H.; Huo, S.D.; Wen, T.Y.; Liu, W.J. Rapid identification of legionella pathogenicity by surface-enhanced raman spectroscopy. *Biomed. Environ. Sci.* 2015, 28, 437–444.

Sivanesan, A.; Witkowska, E.; Adamkiewicz, W.; Dziewit, L.; Kaminska, A.; Waluk, J. Nanostructured silver-gold bimetallic sers substrates for selective identification of bacteria in human blood. *Analyst* 2014, 139, 1037–1043.

Deng, H.; Zhang, X.; Kumar, A.; Zou, G.; Zhang, X.; Liang, X.J. Long genomic DNA ampiclons adsorption onto unmodified gold nanoparticles for colorimetric detection of *bacillus anthracis*. *Chem. Commun.* 2013, 49, 51–53.

Kalidasan, K.; Neo, J.L.; Uttamchandani, M. Direct visual detection of salmonella genomic DNA using gold nanoparticles. *Mol. Biosyst.* 2013, 9, 618–621.

Khaliil, M.A.; Azzazy, H.M.; Attia, A.S.; Hashem, A.G. A sensitive colorimetric assay for identification of *acinetobacter baumannii* using unmodified gold nanoparticles. *J. Appl. Microbiol.* 2014, 117, 465–471.
129. Veigas, B.; Pedrosa, P.; Carlos, F.F.; Mancio-Silva, L.; Grosso, A.R.; Fortunato, E.; Mota, M.M.; Baptista, P.V. One nanoprobe, two pathogens: Gold nanoprobe multiplexing for point-of-care. *J. Nanobiotechnol.* 2015, 13, 48. [CrossRef] [PubMed]

130. Pedrosa, P.; Veigas, B.; Machado, D.; Couto, I.; Viveiros, M.; Baptista, P.V. Gold nanoprobe for multi loci assessment of multi-drug resistant tuberculosis. *Tuberculosis* 2014, 94, 332–337. [CrossRef] [PubMed]

131. Li, X.; Kong, H.; Mout, R.; Saha, K.; Moyano, D.F.; Robinson, S.M.; Rana, S.; Zhang, X.; Riley, M.A.; Rotello, V.M. Rapid identification of bacterial biofilms and biofilm wound models using a multichannel nanosensor. *ACS Nano* 2014, 8, 12014–12019. [CrossRef] [PubMed]

132. Luo, P.; Liu, Y.; Xia, Y.; Xu, H.; Xie, G. Aptamer biosensor for sensitive detection of toxin a of *Clostridium difficile* using gold nanoparticles synthesized by bacillus stearothermophilus. *Biosens. Bioelectron.* 2014, 54, 217–221. [CrossRef] [PubMed]

133. Luo, P.; Liu, Y.; Xia, Y.; Xu, H.; Xie, G. Aptamer biosensor for sensitive detection of toxin a of *Clostridium difficile* using gold nanoparticles labeled with gold nanoparticles. *Electrophoresis* 2014, 36, 457–466. [CrossRef] [PubMed]

134. Pohlmann, C.; Dieser, I.; Sprinzl, M. A lateral flow assay for identification of *Escherichia coli* by ribosomal RNA hybridisation. *Analyst* 2014, 139, 1063–1071. [CrossRef] [PubMed]

135. Chang, Y.C.; Yang, C.Y.; Sun, R.L.; Cheng, Y.F.; Kao, W.C.; Yang, P.C. Rapid single cell detection of staphylococcus aureus by aptamer-conjugated gold nanoparticles. *Sci. Rep.* 2013, 3, 1863. [CrossRef] [PubMed]

136. Veigas, B.; Jacob, J.M.; Costa, M.N.; Santos, D.S.; Viveiros, M.; Inacio, J.; Martins, R.; Barquinha, P.; Fortunato, E.; Baptista, P.V. Gold on paper-paper platform for Au-nanoprobe TB detection. *Lab Chip* 2012, 12, 4802–4808. [CrossRef] [PubMed]

137. Zhao, J.; Tang, S.; Storhoff, J.; Marla, S.; Bao, Y.P.; Wang, X.; Wong, E.Y.; Ragupathy, V.; Ye, Z.; Hewlett, I.K. Multiplexed, rapid detection of H5N1 using a pcr-free nanoparticle-based genomic microarray assay. *BMC Biotechnol.* 2010, 10, 74. [CrossRef] [PubMed]

138. Li, X.Z.; Kim, S.; Cho, W.; Lee, S.Y. Optical detection of nanoparticle-enhanced human papillomavirus genotyping microarrays. *Biom. Opt. Express* 2013, 4, 187–192. [CrossRef] [PubMed]

139. Chudobova, D.; Cihalova, K.; Skalickova, S.; Zitka, J.; Rodrigo, M.A.; Milosavljevic, V.; Hynek, D.; Kopel, P.; Vesely, R.; Adam, V.; et al. 3D-printed chip for detection of meticillin-resistant staphylococcus aureus labeled with gold nanoparticles. *Electrophoresis* 2015, 36, 457–466. [CrossRef] [PubMed]

140. Low, K.F.; Rijiravanich, P.; Singh, K.K.; Surareungchai, W.; Yean, C.Y. An electrochemical genosensing assay based on magnetic beads and gold nanoparticle-loaded latex microspheres for vibrio cholerae detection. *J. Biomed. Nanotechnol.* 2015, 11, 702–710. [CrossRef] [PubMed]

141. Ahmed, S.R.; Kim, J.; Suzuki, T.; Lee, J.; Park, E.Y. Detection of influenza virus using peroxidase-mimic of gold nanoparticles. *Biotechnol. Bioeng.* 2016. [CrossRef] [PubMed]

142. Chen, M.; Yu, Z.; Liu, D.; Peng, T.; Liu, K.; Wang, S.; Xiong, Y.; Wei, H.; Xu, H.; Lai, W. Dual gold nanoparticle lateflow immunoassay for sensitive detection of *Escherichia coli* O157:H7. *Anal. Chim. Acta* 2015, 876, 71–76. [CrossRef] [PubMed]

143. Nie, X.M.; Huang, R.; Dong, C.X.; Tang, L.J.; Gui, R.; Jiang, J.H. Plasmonic elisa for the ultrasensitive detection of treponema pallidum. *Biosens. Bioelectron.* 2014, 58, 314–319. [CrossRef] [PubMed]

144. Zhan, L.; Zhen, S.J.; Fan, Q.; Zhang, Y.; Wang, S.; Zeng, T. Silver nanoparticles coated graphene electrochemical sensor for the ultrasensitive analysis of avian influenza virus H7. *Anal. Chim. Acta* 2016, 913, 121–127. [CrossRef] [PubMed]

145. Wang, Z.; Miu, T.; Xu, H.; Duan, N.; Ding, X.; Li, S. Sensitive immunoassay of listeria monocytogenes with highly fluorescent bioconjugated silica nanoparticles probe. *J. Microbiol. Methods* 2010, 83, 179–184. [CrossRef] [PubMed]
149. Chen, X.; Wu, X.; Gan, M.; Xu, F.; He, L.; Yang, D.; Xu, H.; Shah, N.P.; Wei, H. Rapid detection of staphylococcus aureus in dairy and meat foods by combination of capture with silica-coated magnetic nanoparticles and thermophilic helicase-dependent isothermal amplification. J. Dairy Sci. 2015, 98, 1563–1570. [CrossRef] [PubMed]

150. Huh, A.J.; Kwon, Y.J. “Nanoantibiotics”: A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J. Control. Release 2011, 156, 128–145. [CrossRef] [PubMed]

151. Beyth, N.; Houri-Haddad, Y.; Domb, A.; Khan, W.; Hazan, R. Alternative antimicrobial approach: Nano-antimicrobial materials. Evid. Based Complement. Altern. Med. 2015, 2015, 246012. [CrossRef] [PubMed]

152. Yah, C.S.; Simate, G.S. Nanoparticles as potential new generation broad spectrum antimicrobial agents. Daru 2015, 23, 43. [CrossRef] [PubMed]

153. Hussain, S.M.; Hess, K.L.; Gearhart, J.M.; Geiss, K.T.; Schlager, J.J. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol. In Vitro 2005, 19, 975–983. [CrossRef] [PubMed]

154. Mahmoudi, M.; Serpooshan, V. Silver-coated engineered magnetic nanoparticles are promising for the success in the fight against antibacterial resistance threat. ACS Nano 2012, 6, 2656–2664. [CrossRef] [PubMed]

155. Gupta, A.; Landis, R.F.; Rotello, V.M. Nanoparticle-based antimicrobials: Surface functionality is critical. F1000Research 2016, 5. [CrossRef] [PubMed]

156. Pelgrift, R.Y.; Friedman, A.J. Nanotechnology as a therapeutic tool to combat microbial resistance. Adv. Drug Deliv. Rev. 2013, 65, 1803–1815. [CrossRef] [PubMed]

157. Hameed, A.S.; Karthikeyan, C.; Ahamed, A.P.; Thajuddin, N.; Alharbi, N.S.; Alharbi, S.A.; Ravi, G. In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae. Sci. Rep. 2016, 6, 24312. [CrossRef] [PubMed]

158. Dastjerdi, R.; Montazer, M. A review on the application of inorganic nano-structured materials in the modification of textiles: Focus on anti-microbial properties. Colloids Surf. B Biointerfaces 2010, 79, 5–18. [CrossRef] [PubMed]

159. Grumezescu, A.M.; Gestal, M.C.; Holban, A.M.; Grumezescu, V.; Vasile, B.S.; Mogosanu, L.; Iordache, F.; Bleotu, C.; Mogosanu, G.D. Biocompatible Fe$_3$O$_4$ increases the efficacy of amoxicillin delivery against gram-positive and gram-negative bacteria. Molecules 2014, 19, 5013–5027. [CrossRef] [PubMed]

160. Gestal, M.C.; Holban, A.M. Advances in nanotechnology as an alternative against superbugs. JSM Chem. 2014, 2, 1–5.

161. Saidykhan, L.; Abu Bakar, M.Z.; Rukayadi, Y.; Kura, A.U.; Latifah, S.Y. Development of nanoantibiotic delivery system using cockle shell-derived aragonite nanoparticles for treatment of osteomyelitis. Int. J. Nanomed. 2016, 11, 661–673. [CrossRef] [PubMed]

162. Dhanalakshmi, V.; Nimal, T.R.; Sabitha, M.; Biswas, R.; Jayakumar, R. Skin and muscle permeating antibacterial nanoparticles for treating staphylococcus aureus infected wounds. J. Biomed. Mater. Res. B Appl. Biomater. 2016, 104, 797–807. [CrossRef] [PubMed]

163. Bolocan, A.; Mihaiescu, D.E.; Andronescu, E.; Voicu, G.; Grumezescu, A.M.; Ficai, A.; Vasile, B.S.; Bleotu, C.; Chifiriuc, M.C.; Pop, C.S. Biocompatible hydrodispersible magnetite nanoparticles used as antibiotic drug carriers. Rom. J. Morphol. Embryol. 2015, 56, 365–370. [PubMed]

164. Min, J.; Choi, K.Y.; Dreaden, E.C.; Padera, R.F.; Braatz, R.D.; Spector, M.; Hammond, P.T. Designer dual therapy nanolayered implant coatings eradicate biofilms and accelerate bone tissue repair. ACS Nano 2016, 10, 4441–4450. [CrossRef] [PubMed]

165. Milo, S.; Thet, N.T.; Liu, D.; N zakizwanayo, J.; Jones, B.V.; Jenkins, A.T. An in-situ infection detection sensor coating for urinary catheters. Biosens. Bioelectron. 2016, 81, 166–172. [CrossRef] [PubMed]

166. Diab, R.; Khameneh, B.; Joubert, O.; Duval, R. Insights in nanoparticle-bacterium interactions: New frontiers to bypass bacterial resistance to antibiotics. Curr. Pharm. Des. 2015, 21, 4095–4105. [CrossRef] [PubMed]