Two-Dimensional Nanomaterials for Biomedical Applications: Emerging Trends and Future Prospects

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Two-dimensional (2D) nanomaterials are ultrathin nanomaterials with a high degree of anisotropy and chemical functionality. Research on 2D nanomaterials is still in its infancy, with the majority of research focusing on elucidating unique material characteristics and few reports focusing on biomedical applications of 2D nanomaterials. Nevertheless, recent rapid advances in 2D nanomaterials have raised important and exciting questions about their interactions with biological moieties. 2D nanoparticles such as carbon-based 2D materials, silicate clays, transition metal dichalcogenides (TMDs), and transition metal oxides (TMOs) provide enhanced physical, chemical, and biological functionality owing to their uniform shapes, high surface-to-volume ratios, and surface charge. Here, we focus on state-of-the-art biomedical applications of 2D nanomaterials as well as recent developments that are shaping this emerging field. Specifically, we describe the unique characteristics that make 2D nanoparticles so valuable, as well as the biocompatibility framework that has been investigated so far. Finally, to both capture the growing trend of 2D nanomaterials for biomedical applications and to identify promising new research directions, we provide a critical evaluation of potential applications of recently developed 2D nanomaterials.

1. Introduction

Although graphene was assumed for decades to be thermodynamically unstable and impossible to isolate, the advent of isolated graphene layers sparked an explosion of interest in two-dimensional (2D) nanomaterials.[1] Graphene was quickly shown to have exceptional mechanical strength, high thermal conductivity, and unusual electrical properties, including high conductivity and charge carrier mobility, all stemming from its 2D structure. One feature that stood out to researchers was that graphene's properties were drastically different from the 0D, 1D, and 3D forms of carbon. Just a few years after graphene was first isolated, dimensionality is recognized as one of the most important and influential material parameters of nanomaterials.[2] Graphene's unprecedented properties sparked a search for additional 2D nanomaterials with their own unique properties. This search has led to dozens of 2D nanomaterials being reported in the past few years including synthetic silicate clays, layered double hydroxides (LDHs), transition metal dichalcogenides (TMDs), transition metal oxides (TMOs), and other types of 2D nanomaterials (**Figure 1**). 2D nanomaterials are defined as particles with one dimension that is confined to the nanometer length scale (<100 nm). Due to their unique shape, they have high surface-to-volume ratios as well as anisotropic physical and chemical properties compared to 3D nanomaterials.

2D nanomaterials are highly diverse in terms of their mechanical, chemical, and optical properties, as well as in size, shape, biocompatibility, and degradability. These diverse properties make 2D nanomaterials suitable for a wide range of applications, including drug delivery, imaging, tissue engineering, and biosensors, among others.[3] However, their low-dimensional nanostructure gives them some common characteristics. For example, 2D nanomaterials are the thinnest materials known, which means that they also possess the highest specific surface areas of all known materials. This characteristic makes these materials invaluable for applications requiring high levels of surface interactions on a small scale. As a result, 2D nanomaterials are being explored for use in drug delivery systems, where they can adsorb large numbers of drug molecules and enable superior control over release kinetics. Additionally, their exceptional surface area to volume ratios and typically high modulus values make them useful for improving the mechanical properties of biomedical nanocomposites, even at low concentrations. Their extreme thinness has been instrumental for breakthroughs in biosensing and gene sequencing. Moreover, the thinness of these molecules allows them to respond rapidly to external signals such as light, which has led to utility in optical therapies of all kinds, including imaging applications, photothermal therapy (PTT), and photodynamic therapy (PDT).

Despite the rapid pace of development in the field of 2D nanomaterials, these materials must be carefully evaluated for biocompatibility in order to be relevant for biomedical applications. The newness of this class of materials means that even the relatively well-established 2D materials like graphene are poorly understood in terms of their physiological

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interactions with living tissues. Additionally, the complexities of variable particle size and shape, impurities from manufacturing, and protein and immune interactions have resulted in a patchwork knowledge of the biocompatibility of these materials.

Unfortunately, the cyto- and biocompatibility of 2D nanoparticles cannot be inferred from the corresponding bulk material, as size and shape significantly affect the body's interactions with the material. Additionally, it should be noted that among the various articles reporting the benefits of nanomaterials for biomedical use, there is a notable scarcity of toxic reactions reported. The primary mechanism of harm that nanomaterials have been suggested to cause is through oxidative damage from free radicals. Oxidative damage may be the result of immune responses elicited by the material, the presence of oxidizing contaminants, or from intrinsic properties of the molecules themselves or their degradation products. Additionally, the slow clearance of some nanoparticles by the body may result in particle accumulation in the liver, kidneys, spleen, or lungs. Damage has also been suggested to occur via apoptosis, hemolysis, or thrombosis. Some 2D nanomaterials also contain metals not usually found above trace levels in humans. Again, however, none of these mechanisms can be generalized, as toxicity has been shown to depend on nanomaterial size, surface area, and composition. Size and shape might affect toxicity by making phagocytosis by macrophages impossible, or by allowing nanoparticle aggregates to form. Surface area increases the material's ability to interact with the body, which could increase immunogenicity. The composition of nanomaterials can obviously affect biocompatibility. However, it can also affect protein adsorption on the surface of the nanomaterial. It is well established in the biomaterials community that protein adsorption is rapid in vivo and that this process drives the biological response to implanted materials.[4] However, protein adsorption onto nanomaterial surfaces, which will depend on both the chemical composition and the location of the nanomaterial, remains uncharacterized.

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In comparison to other types of nanomaterials, 2D nanomaterial safety information for materials besides graphene is practically nonexistent. No systematic evaluation of the biocompatibility of any 2D nanomaterial has been completed. With that in mind, preliminary reports have indicated that some of the 2D nanomaterials are highly biocompatible in vitro and in vivo. These materials have been shown to not cause significant harm in individual, small-scale studies. As a result, each of these materials is being regarded with cautious optimism in terms of its potential in biomedicine. A recent literature search (according to ISI Web of Science, April 2015) indicated immense interest in evaluating the biological properties of different types of 2D nanomaterials for biomedical applications including tissue engineering, cancer therapy and drug delivery, biosensors, and bioimaging

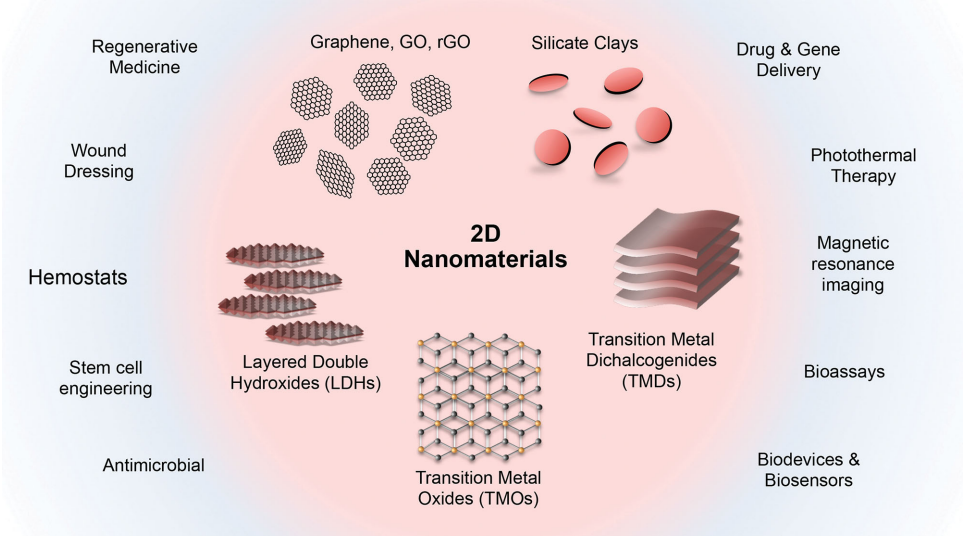

Figure 1. Two-dimensional (2D) nanomaterials investigated for biological applications include carbon-based nanomaterials (graphene, graphene oxide (GO) and rGO), silicate clays, layered double hydroxides (LDHs), transition metal dichalcogenides (TMDs) and transition metal oxides (TMOs).

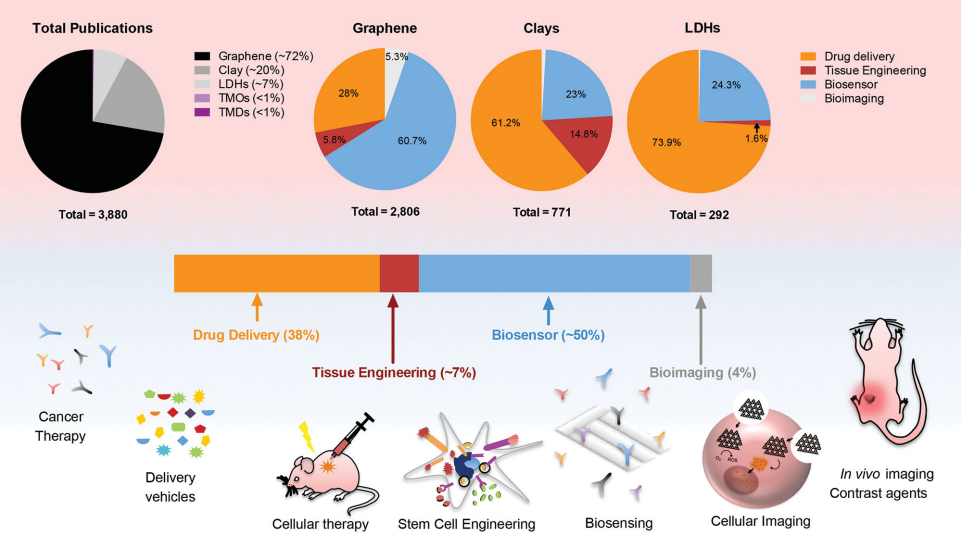

Figure 2. Current research trends in 2D nanomaterials and some of their promising biomedical applications. A recent surge in 2D nanomaterials research is evident from the number of publications in the last few years. The publication data was obtained from ISI Web of Science in April 2015. Carbon-based 2D nanomaterials are being extensively investigated for biomedical applications, followed by clay-based nanomaterials and LDHs. Only a few reports have focused on the biomedical applications of TMOs and TMDs. Most of the biomedical applications of 2D nanomaterials have been in the areas of biosensors and drug delivery, followed by tissue engineering and bioimaging.

(**Figure 2**). This high level of interest clearly indicates that 2D nanomaterials are an emerging material technology that has transformative potential for biomedical and biotechnological innovation.

2D nanomaterials research is still in its infancy, with the bulk of research focusing on elucidating the unique material properties of 2D nanomaterials. Recently, a range of review articles on 2D nanomaterials have been published that focus on the synthesis and molecular assembly of 2D nanomaterials[5] and highlight their fundamental characteristics and properties, which stem from their unique structures, for electronics applications.[6,7] Nevertheless, translational research involving these materials has expanded dramatically. Some of the focused review articles provide a close look at particular subsets of 2D nanomaterials and applications.[8,9] To date, however, there has not been a review that encompasses all biomedical 2D nanomaterials research and provides a systematic overview of the field and its recent developments and direction, and that compares the emerging biomedical applications of each family of 2D nanomaterials.

In this review, we focus on state-of-the-art biomedical applications of 2D nanomaterials, highlight recent developments that are shaping this emerging field, and evaluate the potential applications of recently developed 2D nanomaterials. The discussion is limited to the most promising nanoparticles from each family of 2D nanoparticles (carbon-based, clays, LDHs, TMDs, TMOs, and other types of 2D nanomaterials) that are relevant for biomedical and biotechnological applications. The scope of this paper is to capture the current state of 2D nanomaterial research for biomedical applications and to identify promising new research directions in the field. Additionally, we will review the unique characteristics that make 2D nanoparticles such exciting and useful materials.

2. Structures of 2D Nanomaterials

The physical, chemical and biological properties of nanomaterials strongly depend on their atomic arrangements. 2D nanomaterials are unique compared to other types of nanomaterials because one of their dimensions is only a few atomic layers thick (**Figure 3**).[10] Graphene is the archetypal 2D nanomaterial and exhibits many of the structural motifs that define this category of nanomaterials. The structure of graphene is a single monolayer of carbon atoms that are bonded together via covalent sp^2 bonds in a flat and regular hexagonal pattern. In contrast, graphene oxide (GO) is based on the same regular hexagonal pattern of carbon atoms, but instead of being entirely composed of sp^2 bonded carbon atoms, it has frequent sp^3 carbons bound to functional groups above or below the plane of the nanomaterial. This makes GO less flat than graphene and results in significant local polarity of the structure. Reduced graphene oxide (rGO) is a structural intermediate between graphene and GO. It can be synthesized by the reduction of GO via various methods, which remove most of the functional groups and partially restore the sp^2 hybridization. The result is a sparsely functionalized graphene monolayer with a higher concentration of structural defects than graphene.[11]

Other 2D nanomaterials with structures similar to graphene include silicene, germanene, hexagonal boron nitride (hBN), and graphitic carbon nitride (C_3N_4). Silicene and germanene are 2D allotropes of silicon and germanium, respectively, with buckled, rather than flat, monolayers. C_3N_4 on the other hand, is an alternating monolayer of carbon and nitrogen atoms. Similarly, hexagonal boron nitride (hBN) is composed of covalently bound alternating nitrogen and boron atoms.

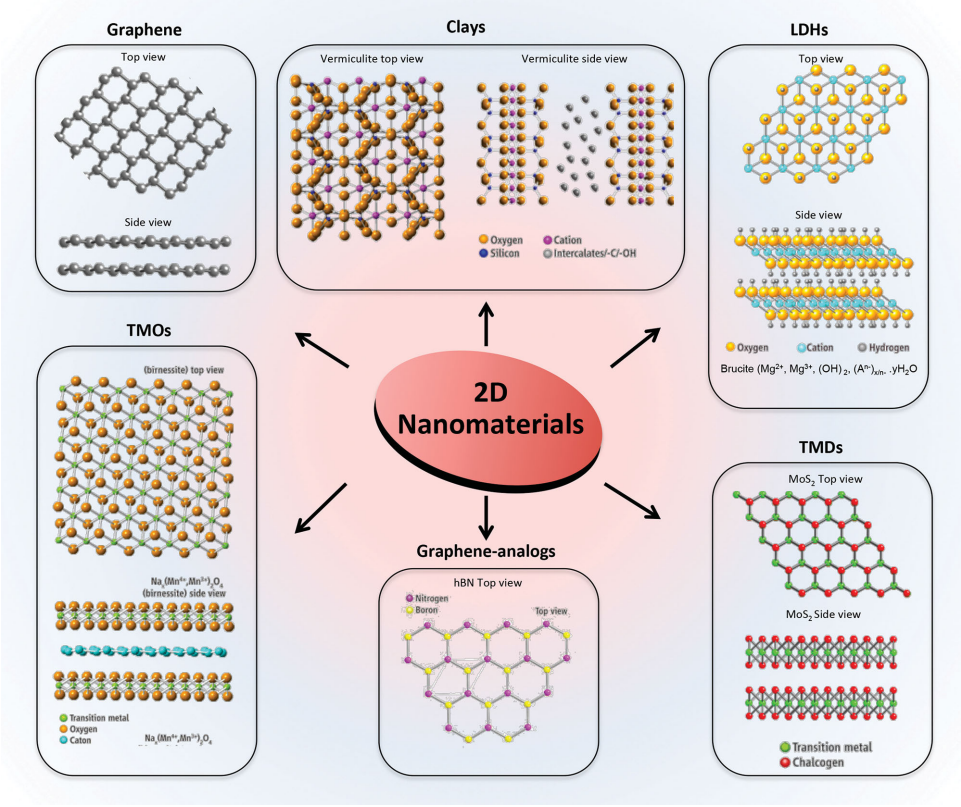

Figure 3. Structures of 2D nanomaterials highlighting a nanosheet network in which one of the dimensions is only a few atomic layers thick. Structures for graphene, clays, LDHs, TMOs, TMDs, and graphene-analogs (hBN) are illustrated here. Adapted with permission.[10] Copyright 2013, American Association for the Advancement of Science.

In contrast to 2D nanomaterials of a monoatomic thickness, some materials like 2D clays, LDHs, TMOs, and TMDs are composed of stable, single crystal units. Laponite, for example, is a 2D nanoclay with 3 layers comprising 2 tetrahedral silica sheets sandwiching an interior octahedral layer of magnesium and lithium cations. Substitutions and edge valences give these nanoparticles permanent negative face charges and positive edge charges, both of which can be stabilized by ionic interactions. Individual laponite nanoparticles are typically disc shaped, with a diameter of roughly 30 nm and a thickness of less than 1 nm.[7,8] LDHs are also called anionic clays; they have positively charged faces, which is a rare quality relative to negatively charged faces.[12] LDHs are structurally similar to brucite, the mineral form of magnesium hydroxide, and consist of magnesium cations surrounded octahedrally by hydroxide ions, but with partial Al^{3+} substitution for Mg^{2+}, resulting in a positive surface charge.[12,13]

2D TMOs can have different structures depending on their individual components. MgO_2 and TiO_2, which are the most commonly used, generally have octahedral conformations. Similar to clays and LDHs, they often exist as stacks with interlayer ions holding these stacks together. 2D TMO nanoparticles are less than 1 nm thick but have been synthesized up to widths of 100 microns.[14] 2D TMDs have a three layer atomic structure where the outside layers are chalcogens covalently bonded to a metal atom inner layer. Each of these layers is in a triangular lattice structure. This crystal structure forms a 2D hexagonal lattice alternating between chalcogenide and metal atoms. TMD monolayers are roughly 0.6 nm thick.[7,15]

3. Carbon-Based 2D Nanomaterials for Biomedical Applications

Graphene is the best-researched as well as the oldest 2D nanomaterial, first being isolated in 2004.[1] The application of graphene in tissue engineering expanded swiftly due to its unprecedented mechanical strength, electrical conductivity, biocompatibility, and thermal conductivity.[16] Additionally, graphene has a higher specific surface area, lower production and purification costs, and greater ease of functionalization compared to its 1D counterpart, the carbon nanotube. Graphene is often partially oxidized into graphene oxide (GO) in order to increase its hydrophilicity and enable facile functionalization, but this modification comes at the expense of electrical conductivity.[17] Reduced graphene oxide (rGO), which can be easily produced in large quantities from GO, is often used as a substitute for pure graphene due to its lower cost, but it has inferior properties due to structural defects.[18,19] The graphene family is therefore composed of 3 materials: graphene, GO, and rGO. As numerous reviews are available on graphene-based materials for biomedical applications,[16,18] we will highlight only some representative recent examples in the areas of tissue engineering, drug delivery and biosensing.

3.1. Graphene

One promising future avenue for graphene-based biomaterials is in the area of tissue engineering. Recently, Qui et al. synthesized a nanocomposite aerogel with a highly interconnected architecture from poly(*N*-isopropylacrylamide) (PNIPAM) and graphene.[20] The graphene aerogel exhibited one order of magnitude higher modulus compared to graphene-free PNIPAM hydrogels. The addition of graphene also significantly improved the electrical conductivity and thermo-responsive properties of nanocomposite hydrogels compared to PNIPAM hydrogels. This effect was mostly attributed to the prefabrication of a graphene aerogel, which ensured high connectivity between graphene sheets. Moreover, building the nanocomposite from an aerogel also obviated the need to functionalize the graphene into GO for dispersal into solution. Using this approach, graphene could be used in place of less conductive GO for a wide range of applications, potentially leading to a new generation of graphene nanocomposites with superior electrical and mechanical properties.

Graphene is able to increase the mechanical strength and stiffness of hydrogel scaffolds without compromising cytocompatibility, and it has been shown to accelerate the adhesion, proliferation, and differentiation of human mesenchymal stem cells (hMSCs) toward an osteogenic cell fate.[21] Nayak et al. showed that in the presence of osteogenic medium, graphene coating enhances the differentiation of hMSCs (**Figure 4**).[21] The ability of graphene to promote the differentiation of hMSCs has been attributed to its ability to adsorb proteins and bioactive molecules such as dexamethasone and β-glycerophosphate.[22] In another study, graphene was used to engineer 3D porous scaffolds for osteogenic differentiation of hMSCs for bone regeneration.[23] A 3D graphene foam structure was fabricated using a temporary scaffold that was fully removed via $FeCl_3$ etching. These graphene foams were capable of inducing osteogenic differentiation of hMSCs without any osteoinductive growth factors. The ability of graphene foams to induce osteogenic differentiation was attributed to the high mechanical stiffness of the foam, as hMSCs are known to respond to high stiffness environments by differentiating into osteoblasts.[23]

Due to the high electrical conductivity of graphene-based nanocomposites, these materials are being explored for tissue engineering and biosensing applications that require electrical stimulation for functioning. For example, in a recent study Tang et al. engineered an electrically conductive graphene substrate to direct cell fate by increasing electrical interactions between neural stem cells (NSCs).[24] They observed a nearly two-fold increase in the neuron density and excitability (as measured by spontaneous spikes in calcium ions) on a graphene substrate

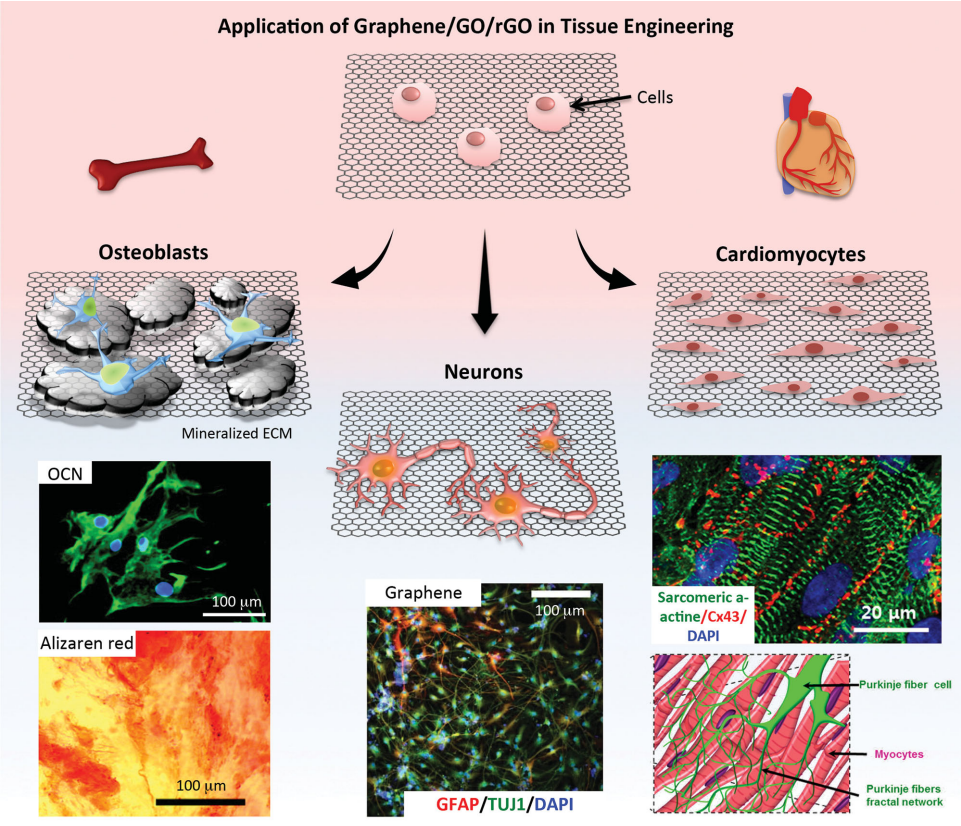

Figure 4. Application of carbon-based 2D nanomaterials for tissue engineering. Graphene/GO/rGO have been used to control and direct cellular fate towards osteoblasts,[21] neurons[24] and cardiomyocytes.[30] Osteoblasts images adapted with permission.[21] Copyright 2011, American Chemical Society. Neurons images adapted with permission.[24] Copyright 2013, American Chemical Society. Cardiomyocytes images adapted with permission[30] Copyright 2013 Elsevier Inc.

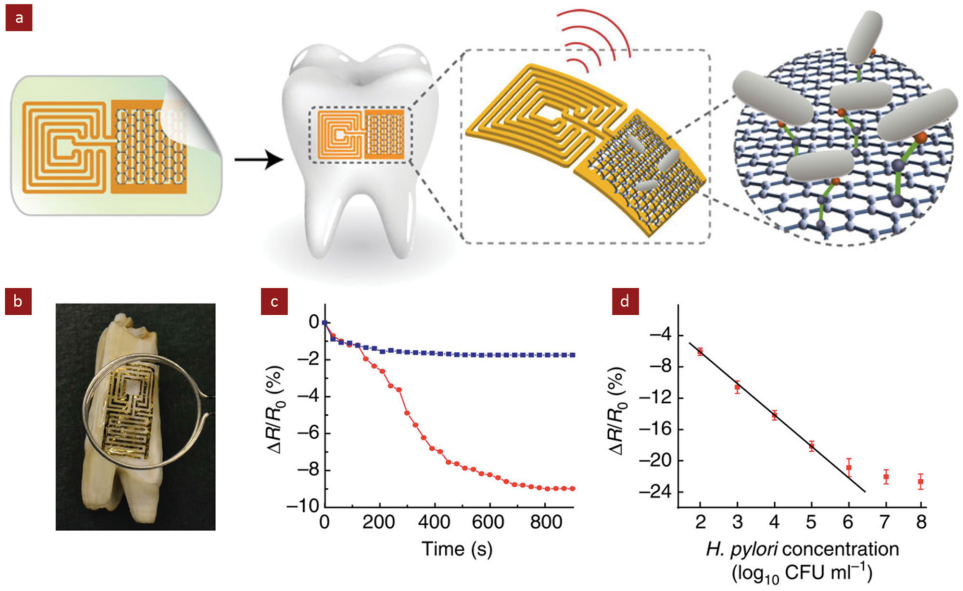

Figure 5. Graphene-based biosensors have been designed for the detection of pathogens. a,b) Graphene-based wireless biosensors printed onto bioresorbable silk can be transferred onto the surface of a tooth to detect binding of bacteria. c) The changes in graphene resistance over time following exposure to *H. pylori* cells in human saliva (red line) were compared to 'blank' saliva (blue line). d) The changes in graphene resistance versus concentration of pathogen illustrated efficacy of a graphene-based biosensor. Reproduced with permission.[25] Copyright 2012, Macmillan Publishers Limited.

compared to tissue culture polystyrene (control). This improvement in NSC differentiation was credited to the high electrical conductivity of graphene, making it a promising substrate for both culturing neurons and for creating biocompatible neural interfaces (Figure 4).[24]

In another study, a graphene-based biosensor was developed to detect bacterial binding on tooth enamel.[25] Mannoor et al. printed a graphene biosensor on a bioresorbable silk substrate and transferred it to a tooth surface.[25] Self-assembly and disassembly of antimicrobial peptides on the graphene surface was used to detect the presence of pathogens. They showed that by coupling this peptide-graphene nanosensor with a resonant coil, it was possible to transmit the signal for wireless detection (**Figure 5**). Overall, these studies highlight the application of graphene-based biomaterials for tissue engineering and biosensing applications.

3.2. Graphene Oxide (GO)

The oxidation of graphene to GO via oxidative exfoliation (Hummer's method) reduces the electrical conductivity and mechanical strength of the material but also makes it more suitable for biomedical applications by effectively rendering the material hydrophilic. This process facilitates material interactions with biomacromolecules such as proteins and extracellular matrix (ECM) components as well as certain drugs. Moreover, GO functionalization, which can enhance cyto- and biocompatibility, is facile due to the presence of hydroxyl groups.[26] One useful characteristic of GO nanosheets is that they repel each other with variable force depending on pH. This electrostatic property can be exploited to create pH-responsive nanocomposites. Bai et al. synthesized a GO-poly(vinyl alcohol) nanocomposite for controlled drug release.[27] Under acidic conditions, the nanocomposite remained solid and was able to retain vitamin B12, the model drug. However, under alkaline conditions, the nanocomposites dissolved rapidly, resulting in rapid release of the entrapped drug. This triggered release was due to the ionization of the carboxyl groups present on GO. The high specific surface area of GO also significantly reduced drug diffusion from the intact nanocomposite. These self-assembling networks could potentially be useful for pH-triggered drug delivery, particularly for oral delivery of acid sensitive drugs.[27,28]

Due to its sheet-like structure and the presence of hydroxyl groups on its surface, GO interacts with a range of synthetic and natural polymers and provides physical reinforcement.[16] Moreover, due to its high electrical conductivity, GO can be used to engineer electrically conductive patches for tissue engineering applications (Figure 4).[29,30] In a recent study, the addition of GO to methacrylated gelatin was used to enhance the electrical conductivity of nanocomposite hydrogels.[29] The enhanced electrical conductivity resulted in higher proliferation of cardiomyocytes seeded on the hydrogel surface, suggesting that this material could be used as a cardiac patch.[29,30] In a separate study, to increase the hydrogel stiffness, the surface of GO was modified with methacrylate groups to covalently crosslink the surface of the GO to the methacrylated gelatin during polymerization.[31] A two- to three-fold increase in mechanical stiffness was observed upon addition of a small amount (3 mg mL^{-1}) of GO to the gelatin hydrogels. Importantly, these nanocomposite scaffolds supported increased cell viability, proliferation, and spreading in a 3D environment.

The large surface area, aromatic structure, and functional groups make GO well suited as a nanocarrier for stimuli-responsive nanocomposites for drug delivery.[32] Conducting

polymers such as polypyrrole (PPy) have been used as electrically-responsive drug delivery systems but have limited loading capacity. In a recent study, Weaver et al. developed electrically responsive GO–PPy nanocomposites for controlled drug delivery.[33] The GO–PPy nanocomposites could entrap and release two-fold more dexamethasone, an anti-inflammatory molecule, relative to pure PPy. Additionally, incorporation of GO resulted in enhanced sensitivity of the nanocomposites to electrical stimulation and allowed for linear release kinetics over 400 stimulation cycles without any measurable drug release in the absence of stimulation. Drug loading and release rates were also shown to be tunable by changing the sonication time of GO. The significant increase in drug loading and the improved release kinetics were attributed to the high surface area of GO. The significant control over the release kinetics, drug loading capacity, and ability to respond to external stimulation to release entrapped drug could be used for a range of biomedical applications including cancer treatment, immunotherapies, and tissue engineering.

GO-loaded hydrogels can also be used for gene delivery for the treatment of myocardial infarction. Paul et al. decorated the surface of GO with polyethylenimine (PEI) to enhance the loading and delivery of a VEGF DNA plasmid (**Figure 6**).[34] They engineered an injectable hydrogel loaded with functionalized GO for minimally invasive therapy. The results indicated that use of functionalized GO for gene delivery reduced in vivo scar formation compared to VEGF plasmid and hydrogel groups, highlighting the potential of GO as a gene delivery agent.

Although GO is less conductive than graphene, its bioactivity and electrical conductance make it a promising new dopant for electrically-responsive nanocomposites for neural interfaces.[35] Poly(3,4-ethylene dioxythiophene) (PEDOT) has been extensively studied for use in neural interfacing due to its biocompatibility and electrical conductance, but success has been limited due to a lack of functional groups on the PEDOT backbone for anchoring biomolecules. To address this limitation, Luo et al. developed GO–PEDOT nanocomposites with improved bio-interfacing.[36] The electrical conductivity and ability of GO to promote neural outgrowth make it an obvious choice for doping PEDOT while preserving its electrical conductivity. The GO–PEDOT nanocomposite retained its conductivity and allowed for covalent conjugation of peptides on its surface. The addition of GO significantly improved neuron outgrowth and attachment on GO–PEDOT surfaces relative to the current standard, PEDOT–PSS. The surface of GO has a high density of carboxyl groups, allowing it to conjugate multiple biomolecules, thus providing a suitable surface for enhanced interactions with neurons. Overall, graphene-based nanocomposites are emerging as promising materials for neural applications, and several exciting approaches are currently being explored.[16,21,36,37]

3.3. Reduced Graphene Oxide (rGO)

Reduced graphene oxide is commonly produced from GO, which takes advantage of the ease of fabrication of GO, and

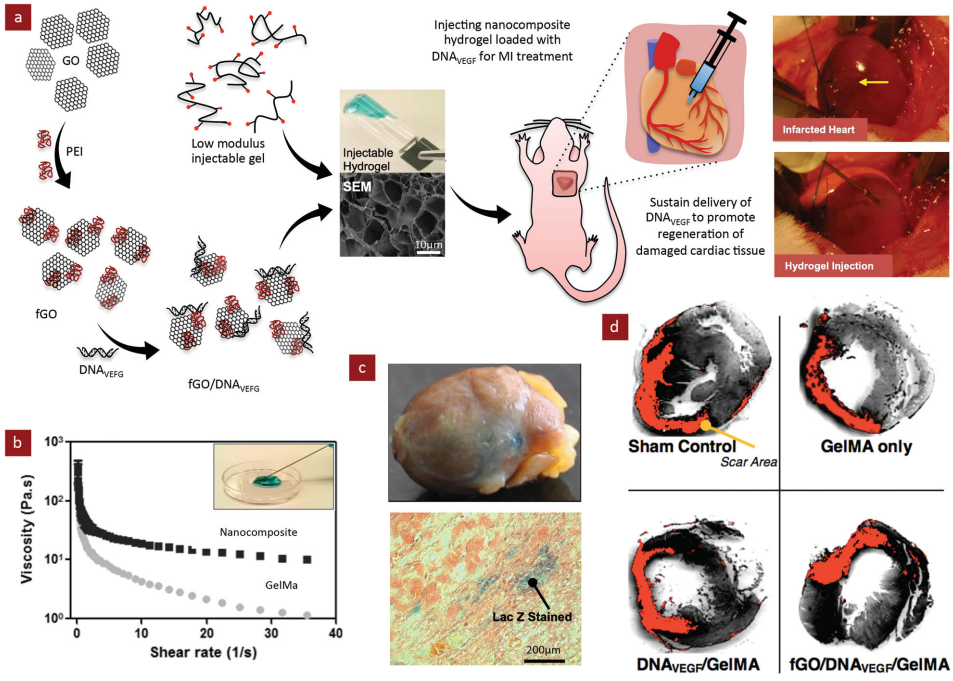

Figure 6. Nanocomposite hydrogel loaded with functionalized GO (fGO) for gene delivery. a) GO was functionalized with cationic polyethylenimine (PEI) to hold anionic DNA$_{VEGF}$ plasmids. fGO/DNA$_{VEGF}$ with the plasmid physically adsorbed on the fGO surface was incorporated within a prepolymer solution of GelMA and then lightly crosslinked to obtain low modulus injectable hydrogels. Photocrosslinked hydrogels loaded with fGO/DNA$_{VEGF}$ were injected into infarcted heart tissue. b) The shear viscosity of fGO/GelMA and GelMA hydrogels indicated that the addition of GO resulted in increased mechanical stiffness. c) The localization of injected gel in the infarcted area was shown by Laz Z staining. d) In vivo scar formation (red area) was reduced with fGO/VEGF plasmid/GelMa treatment. Reproduced with permission.[34] Copyright 2014, American Chemical Society.

exhibits many of the properties of pure graphene sheets. The reduction of GO sheets removes most functional groups from the nanosheets, leaving only the carbon sheet behind, along with some structural defects. rGO has been used extensively in biosensing applications, PDT and PTT due to its enhanced availability and better electrical conductivity compared to GO. Compared to traditional materials like CNTs and silicon nanowires, rGO has higher specific surface area and higher carrier mobility, and has been shown to immobilize high densities of receptor biomolecules on its surface.[38] These features make rGO an attractive choice for high-sensitivity, high-resolution biosensors. For example, rGO has been used to fabricate an ultrasensitive label-free field effect transistor (FET) biosensor that can be used to detect prostate specific antigen at concentrations from 100 fg mL^{-1} up to 10 ng mL^{-1} with high specificity and without the need for labeling. Several studies have created other FET biosensors using rGO for detecting various biomolecules, including DNA and *Escherichia coli* antigens. Importantly, because of their ease of fabrication, low cost, and potential for high sensitivity and specificity, the biosensing technologies being created using rGO could lead to improved diagnostic techniques and, ultimately, better patient outcomes.[38]

rGO-based materials are also being actively researched as PTT agents due to their effectiveness, low cost, and cytocompatibility.[39] GO has previously been used to create similar PTT agents, but high doses and power were required due to GO's poor NIR absorption. rGO absorbs approximately 20% of NIR light, nearly seven-fold more than GO, due to a larger number of π–π bonds.[39] This superior absorptivity allows rGO to be effective at a significantly lower dose compared to GO, CNTs and gold nanoparticles. Functionalization of rGO with ligands for selective cancer cell targeting and doxorubicin delivery has also been demonstrated, suggesting that this nanomaterial may be a candidate for PTT/PDT combined therapy. Notably, rGO is a promising material for PTT because it is comparable in efficacy to gold nanoparticles, is cheaper and easier to mass produce, and can be loaded with chemotherapeutic drugs.[39]

3.4. Multi-Component Carbon-Based Hybrid Nanomaterials

Some researchers have begun experimenting with designing hybrid nanocomposites.[40] Experimental evidence indicates synergistic effects of having multiple types of nanomaterials present within a polymeric structure.[41,42] Various combinations involving multiple dimensionalities of nanomaterials including CNTs, graphene, fullerenes, and nanodiamonds have each resulted in unique synergistic effects. For example, CNT–GO–polypropylene nanocomposites have been shown to possess enhanced mechanical strength, thermal stability, and electrical conductivity compared to two-component nanocomposites, and fullerene–CNT–propylene nanocomposites have also demonstrated improved mechanical and thermal properties.[41] Research indicates that the mechanism of this synergy is multifaceted: one factor that has been identified is that using multiple carbon allotropes enhances the dispersion of nanocomposites throughout the hydrated polymeric network (hydrogel). The allotropes of carbon used for nanocomposites exhibit similar chemical properties, but have very different physical structures, allowing for different hydrogel-nanoparticle bonding patterns within the same structure. Further research is required to fully characterize the mechanism behind the synergistic effects. Hybrid nanocomposites have mainly been researched in the domain of material science, but have clear applications in tissue engineering, and they are likely to expand into tissue engineering applications in the near future. Another example of the use of hybrid nanocomposites is electrospun microfibrous polycaprolactone (PCL) scaffolds loaded with both graphene and single walled CNTs.[43] In Holmes et al., PCL–graphene–single-wall CNT nanocomposites were shown to have higher stiffness, enhanced hMSC proliferation, and enhanced chondrogenic differentiation and collagen II synthesis compared to a pure PCL scaffold.[43] Although this study did not compare the results to single nanomaterial controls, it nevertheless demonstrates the suitability of these hybrid nanocomposites for tissue engineering.

3.5. Summary of Carbon-Based 2D Nanomaterials for Biomedical Applications

Overall, graphene, GO, and rGO, represent the largest group of 2D nanomaterials for biomedical applications. Due to their outstanding physical, chemical, electrical, and biological characteristics, they have been investigated for a wide range of biomedical applications including tissue engineering, drug delivery, bioimaging, and biosensing.[26,44] Whether these materials will continue to be preponderant in these areas remains to be seen, as a new range of 2D nanomaterials with unique property combinations of their own are emerging. Due to the large volume of carbon-based biomedical research, this section has only provided a brief overview of some of the recent and relevant biomedical applications. The main challenges that these materials face include their in vivo safety and biocompatibility. The lack of control over the dimensions of carbon-based 2D nanomaterials makes it difficult to employ a standardized approach to evaluate the cyto- and biocompatibility of these materials and make comparisons with other nanomaterials. Additionally, improved control over nanosheet size, size distribution, and functionalization need to be achieved in order to better understand how these materials interact with biological entities such as proteins, DNA and cells. In biosensing and bioimaging, weak fluorescence and broad emissions are some of the limitations that also need to be addressed before we can utilize this new array of nanomaterials for clinical applications.

4. Silicate Clays as Bioactive Nanomaterials for Biomedical Applications

Silicate clays have been widely used in modern medicine for several decades as antacids and topical creams, but their application as biomaterials remained relatively uninvestigated until recently.[8,45] 2D clay nanoparticles used in biomedical engineering are typically layered silicates that are 10–100 nm in diameter and ≈1 nm in thickness. The most useful clay nanoparticles for biomedical applications also have a distinct layered structure that generates a permanent negative surface

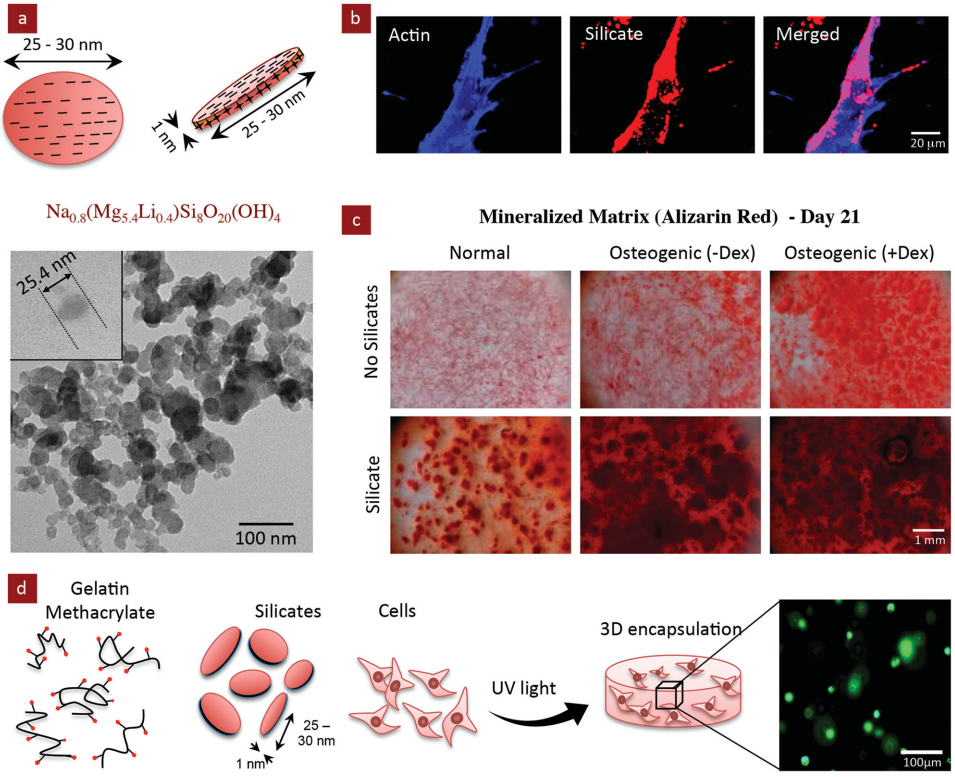

Figure 7. Bioactive silicate nanoclays induce osteogenic differentiation of stem cells and can be used for bone regeneration. a) Schematic and TEM images of silicate nanoparticles showing shape and size. b) Fluorescence imaging demonstrated internalization of silicate nanoparticles within stem cells. c) The effect of silicate nanoparticles on the production of mineralized ECM indicated the osteoinductive properties of silicates. d) Nanocomposite hydrogels for bone regeneration were fabricated by combining photocrosslinkable polymer (GelMA), silicate nanoparticles and stem cells. Panels (a-c) reproduced with permission[55] Copyright 2013, John Wiley & Sons, Inc. and panel (d) reproduced with permission[47] Copyright 2014, American Chemical Society.

charge on each face of the particle and a positive charge along the edges, which gives these nanoparticles high drug loading capacity, aqueous stability, shear thinning characteristics, and enhanced cell-nanomaterial interactions.[45,46] The silicates with this structure are kaolinite, palygorskite, sepiolite, and the smectites (laponite, montmorillonite, saponite, and hectorite).[8] Among these, the smectites are the most extensively investigated for biomedicine applications. In the smectite group of 2D clay nanoparticles, nanoplatelets are composed of a metal cation layer sandwiched between two tetrahedral silica sheets, a 2:1 layer conformation. The nanoparticle surface has a weak net negative charge caused by cationic substitution, and unbalanced charges create a net positive charge on the edges of each nanoparticle. The relative weakness of their surface charges compared to similar clays makes smectites attractive clays for biomedical purposes. Their smaller surface charges make delamination into individual nanoplatelets a facile process that greatly increases surface area and increases nanoscale interactions with other molecules.

The biocompatibility of clay nanoparticles has not been systematically investigated, but is much better understood than that of most other 2D nanomaterials. In vitro and in vivo testing of a smectite clay (Laponite), for example, has demonstrated favorable cyto- and biocompatibility, and in vivo degradation of smectite nanoparticles has been observed.[47,48] Biodegradation of clay nanoparticles is also nearly unique among 2D nanomaterials, as most of the clays are composed of minerals that are already present in body and have been shown to degrade into these nontoxic components under physiological conditions. Thus, their degradation pathway is better understood than other nanomaterials. The ability of silicate nanoplatelets to interact with hMSCs and human adipose stem cells (hASCs) over a period of 28 days was previously investigated (**Figure 7**).[47,49] At a lower concentration of silicate nanoparticles (100 µg mL^{-1}), no significant effects on cellular morphology, proliferation, viability, or the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and lactose dehydrogenase (LDH) were observed, indicating high cyto-compatibility.[49] However, at higher concentrations of silicate nanoparticles, a significant reduction in metabolic activity was observed, with the half-maximum inhibitory concentration (IC_{50}) being ≈4 mg mL^{-1}.[49] When compared to similarly sized carbon-based nanoparticles, silicate nanoparticles were only cytotoxic at a ten-fold higher concentration, indicating comparatively high cytocompatibility.[49] Moreover, these silicate nanoparticles are readily internalized by cells via clathrin-mediated endocytosis due to their ability to interact with proteins and cell surfaces.[50]

Silicate nanoparticles are not only short-term cytocompatible with human cells but they have also been shown to increase both cell adhesion and survival on hydrogel surfaces.[51] In

Liu et al., silicate nanoparticles were shown to improve cell adhesion on a PNIPAm–poly(ethylene glycol) (PEG)–silicate nanocomposite.[8,52] Other studies have shown that silicate nanoparticles promote initial cell adhesion, spreading and proliferation when added to non-fouling surfaces.[53,54] Silicate nanoparticles have also been shown to improve the mechanical stiffness of collagen-based hydrogels by four-fold.[47] These behaviors are all attributed to non-covalent interactions between the charged nanoparticle surfaces and polymer chains. Because these noncovalent interactions can break and re-form, they also impart hydrogel nanocomposites with shear thinning viscoelastic properties that make them well-suited for minimally invasive therapies.[46,53,55] By controlling the interactions between silicate nanoparticles and polymers, highly elastomeric fiber shaped cellular constructs can be fabricated.[56] Additionally, the inclusion of silicate nanoparticles has been shown to increase alkaline phosphatase activity and in vitro matrix mineralization. The ability of silicate nanoparticles to increase bioactivity in other polymers has recently been demonstrated as well. For example, in one recent study silicate nanoparticles were shown to enhance osteogenic differentiation on electrospun polycaprolactone scaffolds.[46,47,55]

Recently, silicate nanoparticles have begun to be investigated for musculoskeletal tissue engineering applications.[8,46] Silicate nanoparticles can stimulate the differentiation of stem cells into osteoblasts and promote production of type I collagen, even in the absence of exogenous growth factors such as bone morphogenic protein 2 (BMP2).[8,46] These particles trigger upregulation of osteogenic genes including alkaline phosphatase, Runt-related transcription factor–2, osteocalcin and osteopontin (Figure 7).[47,49,50] Moreover, with the addition of a small amount of nanoparticles, stem cells produce a significantly higher amount of mineralized matrix compared to stem cells seeded on tissue culture polystyrene. The osteoinductive characteristics of clay nanoparticles have been attributed to their dissolution products—Na^+, Mg^{2+}, $Si(OH)^4$, and Li^+—which might promote osteogenic pathways. In particular, orthosilicic acid ($Si(OH)_4$) upregulates bone-related gene expressions and promotes collagen I synthesis, while Li^+ activates canonical Wnt signaling by inhibiting glycogen synthase kinase–3 beta, which leads to the upregulation of osteogenesis-associated genes such as Runx–2 transcription factor.[57] The ability to stimulate stem cell differentiation without exogenous growth factors represents a potentially important pathway in tissue engineering because the use of growth factors to direct differentiation generally requires supraphysiological doses that may cause serious consequences in vivo.[58] Using growth factor-free approaches like nanosilicates may provide an alternative with fewer side effects.

The well characterized biodegradability and high loading efficiency of silicate nanoparticles have made them a good choice for drug delivery. Silicate nanoparticles can noncovalently adsorb a wide range of biomolecules, including therapeutics.[59] Silicate nanoparticles can also passively target tumors due to the enhanced permeability and retention (EPR) effect, and can be functionalized with biological molecules in order to actively target tumors.[60] In vitro and in vivo experiments with doxorubicin have shown better efficacy than free doxorubicin due to higher cell uptake, and in vivo experiments have confirmed that doxorubicin-loaded silicate nanoparticles target tumor cells via the EPR effect, increase drug uptake by tumor cells, and exhibit pH sensitive drug release. Additionally, mice treated with doxorubicin-loaded silicate nanoparticles had normal blood and serum biochemistry parameters, while free doxorubicin-treated mice showed decreased parameters. These results showed that 2D silicate nanoparticles can be used for drug delivery and can enhance efficacy, reduce systemic toxicity, and increase survival.

Another strategy for improving drug delivery using silicate nanoparticles involves the incorporation of nanosilicates in injectable hydrogels.[59] Embedding silicate nanoparticles in a hydrogel matrix delays the release of drug molecules, creating a desirable sustained release profile. Additionally, silicate-hydrogel nanocomposites exhibit shear-thinning properties that make them particularly well suited for injection applications. In Goncalves et al., alginate-silicate-doxorubicin injectable hydrogels were synthesized for cancer therapeutics. The strong interaction of silicate nanoparticles with both the drug and polymer resulted in the formation of a cohesive hydrogel with reduced burst release, increased drug-loading, and a sustained drug release profile over a period of 11 days at tumor pH ≈6.5. In contrast, alginate-doxorubicin hydrogels immediately released most of the loaded drug within 1–3 days. These results indicated that the injectability of silicate nanocomposites can be exploited for drug delivery purposes by engineering desired release profiles, which is a significant obstacle for contemporary drug delivery vehicles.[59]

One biomedical application to which silicate clays are uniquely suited is hemostasis. Kaolinite has been used to evaluate blood-clotting disorders since the 1950s, and a zeolite product (QuikClot) is approved for controlling hemorrhages.[48,61] Recently, silicate clay nanomaterials have been investigated for improving hemostatics.[48] Most hemostatic agents, including zeolite, are powders, which are only effective on external wounds. 2D silicate clay nanoparticles were incorporated in collagen-based hydrogels to engineer shear-thinning hemostatic gels (**Figure 8**).[48] The shear-thinning nanocomposite gels were shown to be self-healing and were able to regain their mechanical integrity quickly (<10s) after injection. These nanocomposite gels demonstrated a clot time comparable to injected thrombin (the gold standard), a clot strength similar to natural clots, and excellent biocompatibility. The nanocomposite gel achieved 100% survival in test rats with otherwise lethal liver bleeding, and they fully degraded within 28 days, allowing unimpeded healing. This gel can be injected to treat wounds that are unreachable by powders and has a much lower risk of being washed to other sites in the body compared to other injectable hemostatics, making it a promising leap forward in hemostatic technology with the potential to save many lives.

In another approach, nanocomposite hydrogels loaded with silicate nanoparticles have been developed as an immune organoid by mimicking the anatomical microenvironment of lymphoid tissue.[62] The addition of silicate nanoparticles resulted in enhanced stability of the hydrogel network due to ionic crosslinking at physiological conditions. The nanoengineered organoid consisted of primary naïve B cells co-cultured with stromal cells. The presence of silicate nanoparticles was shown to support proliferation and activate the B cells up to 100 times faster compared to 2D culture. It is expected that the ability

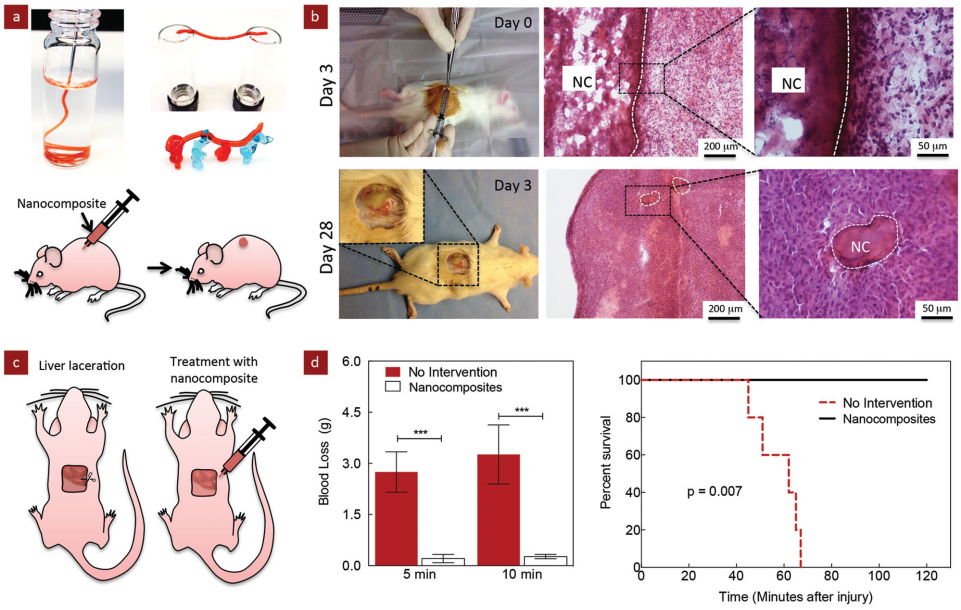

Figure 8. Silicate nanoclays as hemostatic agents. a) The addition of silicate nanoparticles within gelatin resulted in the formation of injectable and self-healing nanocomposite hydrogels. b) Histological staining (hematoxylin and eosin) demonstrated a favorable degradation rate and mild inflammatory response to nanocomposite hydrogels. c) Overview of the surgical method used to test the in vivo efficacy of the nanocomposite hydrogels as a hemostatic agent. d) Results showing that the application of the nanocomposite hydrogel to liver lacerations significantly reduced mortality due to their ability to clot blood. Reproduced with permission.[48] Copyright 2014, American Chemical Society.

to drive germinal center reactions ex vivo at controllable rates will provide the ability to recapitulate immunological events with tunable parameters for better screening and translation of immunotherapeutics.

Overall, 2D silicate clay nanoparticles are being extensively investigated for biomedical applications including controlled cell adhesion, stem cell differentiation, drug/gene delivery, and hemostatic agents. This broad utility is mainly attributed to the shape and surface charge characteristics of these silicate clays, which result in enhanced interactions with biological moieties such as polymers, biomolecules and cells. In the future, we expect to see translational research based on these silicate clays in the fields of tissue engineering, immune modulation, and cancer research.

5. Layered Double Hydroxides (LDHs)

LDHs have been relatively under-researched compared to the other families of 2D nanomaterials, despite having promising applications in biomedical engineering.[63] LDHs consist of an inner layer of cationic metal atoms sandwiched between hydroxide layers. These sheets are found naturally in stacks held together by intercalated anion layers. In contrast to silicate clays, LDH nanoparticles have a higher layer charge density, requiring more chemical modifications or interlayer composition changes to exfoliate individual nanosheets. Until recently, a lack of effective exfoliation techniques hindered the development of LDH nanoplatelet applications compared to clays. Higher charge densities also cause LDH nanoparticles to bind more strongly to anionic moieties. LDH nanoparticles are attracting research interest for drug delivery applications due to their low toxicity and ability to noncovalently bind anionic drug molecules and genetic material.[64] In addition, LDH nanocomposites have been shown to exhibit good mechanical and thermal properties compared to polymeric scaffolds. For example, the addition of LDH nanoparticles to a polymeric sheet results in enhanced mechanical stiffness.[65]

LDHs are highly cytocompatible, have high charge density and anion exchange abilities, and exhibit pH-sensitive drug release.[66] Additionally LDH nanoparticles have been shown to be taken up by cells via clathrin-mediated endocytosis and to be resistant to endosomal effects due to their buffering properties. These properties rank LDH nanoparticles among the most promising candidates for controlled drug delivery. A recent study by Saifullah et al. reported the development of a biocompatible nanodelivery system using LDHs. The tuberculosis drug isoniazid was bound to Mg/Al LDHs and its release kinetics were measured in a simulated buffer solution. The LDHs demonstrated improved cytocompatibility, and drug delivery was more effective compared to free isoniazid when evaluated using normal human lung and murine fibroblast cells. Moreover, the use of LDHs resulted in sustained release kinetics of the entrapped drug.[66]

Another advantage of LDH nanosheets in drug delivery is their ability to be targeted to specific cell types (e.g., cancerous tissue) and their retention in cells.[67] LDHs are notable among drug delivery nanomaterials in their ability to protect drugs from premature release. This can be particularly important for highly toxic drugs like chemotherapeutics. In a recent paper by Ma et al., LDH nanosheets were loaded with cisplatin, and inhibitory concentrations of cisplatin and cisplatin-loaded LDH nanosheets were determined using an array of cancerous and non-cancerous cell lines. This study revealed that the LDH nanosheet delivery

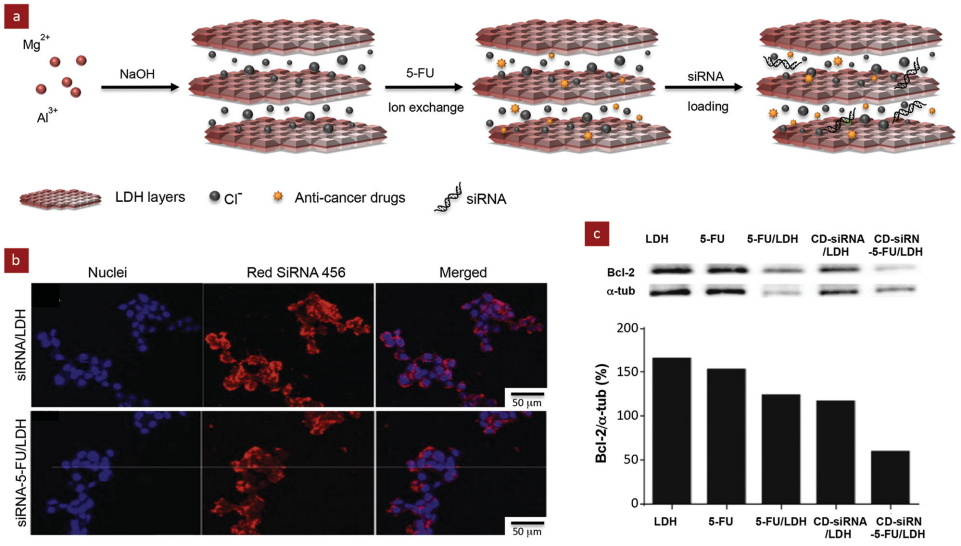

Figure 9. Application of LDHs in drug delivery. a) LDHs bind to negatively charged molecules, including nucleic acids and anionic drugs, due to their high anion exchange capacity. b) Cellular uptake of siRNA/LDH and siRNA/–5–FI/LDHs nanohybrids. c) Suppression of Bcl–2 protein expression in MCF–7 cells after single or combined treatment with 5–FU and CD–siRNA delivered by LDHs. Reproduced with permission.[68] Copyright 2014, Elsevier.

system enhanced cisplatin effectiveness by 11-fold and significantly decreased cytotoxicity to non-cancerous cells. The increase in anti-cancer effectiveness was attributed to increased cellular uptake of the drug-loaded nanoparticles due to endocytosis. Cellular uptake in cancerous cells exposed to the LDHs was increased 15-fold. In contrast, normal cells were unable to uptake LDH and no significant apoptosis was observed. The exact mechanism behind this selectivity is not clear, but this research promises to improve the efficacy of existing chemotherapy drugs.

LDHs have also been used as a combined delivery agent for therapeutics and nucleic acids. In one study focused on evaluating the use of LDHs for dual delivery of drugs and RNA, Li et al. used LDH nanosheets to simultaneously deliver both 5–fluorouracil (5–FU), an anticancer drug, and Allstars Cell Death siRNA (CD–siRNA), a blend of silencing RNAs targeting cell survival genes (**Figure 9**).[68] This approach exploited the anionic exchange capacity of LDH nanosheets to exchange anionic drug molecules and oligonucleotides into interlayer spaces, thereby protecting these molecules during delivery. The results from this study confirmed that the combination therapy results in synergistic cytotoxicity for cancer cells.

Although LDHs are insulators, their biocompatibility, high catalytic activity, high charge density, and strong adsorption have made LDHs an attractive building block for biosensors, where electrical conductance can be handled by another material. For example, Sun et al. fabricated a Mg_2Al/graphene nanocomposite biosensor by combining the excellent adsorption, protective effects, and biocompatibility of LDHs with the high electrical conductivity of graphene.[69] The nanocomposite showed enhanced electrochemical properties and was proven to be a stable and sensitive biosensor. This result was attributed to the ability of LDHs and graphene to facilitate a direct electron transfer process.

Recently, LDHs have also been evaluated as a nanomaterial for creating mechanically stiff nanocomposites for tissue engineering applications. The addition of LDHs to poly(vinyl alcohol) (PVA) resulted in a four-fold increase in tensile strength over pure PVA and an elastic modulus comparable to lamellar bone.[65] The increase in mechanical strength was attributed to a high PVA-LDH interfacial strength owing to the hydrogen bonding interactions between LDH platelets and PVA, which facilitated load transfer between the matrix and nanoparticles. In another study, LDH-based PLGA nanocomposites were designed as guided tissue regeneration membranes for regenerating lost periodontal bone.[70] For periodontal abscesses, guided tissue regeneration (GTR) films have recently been created for surgical implantation between the tooth root and gingiva. In general, GTR films are intended to allow undisturbed repopulation of the tooth root with osteoblasts and periodontal ligament cells. However, most films have only demonstrated limited efficacy due to their lack of bioactivity. Chakraborti et al. designed bioactive PLGA-LDH nanocomposites loaded with alendronate and tetracycline to facilitate periodontal regeneration, and in vitro characterization showed significant increases in both alkaline phosphatase activity and mineralized matrix formation.[70]

Overall, LDHs are a relatively new type of 2D nanomaterial for biomedical applications, but these nanomaterials have excellent biocompatibility, anion exchange capacity, and potential for drug delivery applications. Similar to silicate clay, LDHs can be used for various tissue engineering applications, as most of the components of LDHs are minerals that can be easily absorbed by the body without any significant side effects. Nevertheless, additional studies are required to more thoroughly evaluate the use of LDHs for these applications. We can expect to see the use of LDHs for biomedical applications continue to increase in the coming years.

6. Transition Metal Dichalcogenides (TMDs) for Biomedical Applications

TMDs are 2D nanoparticles that consist of a monolayer of transition metal atoms sandwiched between two layers of chalcogen

atoms (any group 16 element, usually sulfur, selenium, or telluride) in a hexagonal lattice.[7] There are roughly 60 known TMDs. However, only two-thirds of these have layered structures, and among these, only three materials have received significant attention: molybdenum disulfide (MoS_2), tungsten disulfide (WS_2) and titanium disulfide (TiS_2). TMD nanoparticles stand out as biomaterials because of their catalytic properties, photoluminescence, optical absorption, direct bandgap, and high wear resistance.[10,71,72] TMDs are also inherently thin, flexible, and strong. Notably, 2D TMDs are distinguished by a much higher structural rigidity when compared to commonly used 2D nanomaterials like graphene and hexagonal boron nitride (hBN). MoS_2 and WS_2 have flexural rigidities of 27 and 30 eV Å2/atom, respectively, while graphene and hBN are around 3.5 eV Å2/atom. Together, these properties make TMDs useful in biosensors, nanocomposites, bioimaging, and PTT/PDT.

6.1. Molybdenum Disulfide (MoS_2)

As TMD monolayers are fairly new, most research has been on the fundamental characteristics of the materials rather than translational applications. TMDs can be metallic or semimetallic, magnetic or nonmagnetic, have anisotropic thermal conductance, and have exhibited superconducting properties. These characteristics led initial research on TMD applications towards electronics, and indeed 2D TMDs are considered among the most promising building materials for nanoelectronics. One biomedical application of TMDs is in engineering atomically thin nanopores for higher sensitivity DNA translocation.[73–75] Among DNA sequencing technologies, nanopore sequencing is a promising approach to the challenge of sequencing a single DNA molecule. Nanopore sequencing utilizes a nano-size pore that is obstructed to different degrees by each of the four nucleotides as they pass through the pore. Changes in the amount of current that passes through the pore are characteristic of each nucleotide. Until recently, the only way to make nanopores sensitive enough to recognize individual nucleotides was by using membrane protein complexes. Efforts to replace these biopores with solid state nanopores have been stymied by the inability to create nanopores out of SiN_x thin enough to differentiate individual nucleotides. To address this problem Liu et al. used a monolayer or few layers of MoS_2, which can have sub-nanometer thickness.[73] A transmission electron microscope was used to drill through the MoS_2 nanosheets as they were suspended on a 20 nm thick SiN_x membrane. This technique increased the resolution of the solid-state nanopore sequencing from 20 nm to less than 1 nm, breaking down a significant barrier to single nucleotide resolution. The use of TMDs for this biomedical engineering application is important because, unlike 2D graphene and boron nitride, an intrinsic bandgap exists, which opens up the possibility of sequence-specific transistors. Moreover, MoS_2 does not require surface treatments in order to avoid strong interactions with DNA, unlike graphene, and has a wider window of applied voltages. In this way, MoS_2 is being used in the next step towards single molecule sequencing, one of the ultimate goals of the field of DNA sequencing.[73–75]

Other recent biosensing applications have taken advantage of the highly photoluminescent qualities of TMDs. 2D and Quasi-2D MoS_2 nanosheets are photoluminescent nanomaterials, and their photoluminescence (PL) can be controlled via the intercalation of positive ions between the sheets.[72] The PL effect of these materials is the result of the p, s, and d orbitals of the molybdenum atoms in MoS_2. The fewer the number of layers, the better the PL efficiency of the nanoflakes. This property is useful in biomedical applications because it has been shown that the PL of MoS_2 can be controlled by altering electrical gating, light polarization, mechanical stress, and cation intercalation, which causes structural lattice expansion.[72] The PL characteristics of MoS2 are highlighted in **Figure 10**a.[76] The change in PL potential caused by ionic changes can be used as a detector of cation concentrations in biological systems. Cations

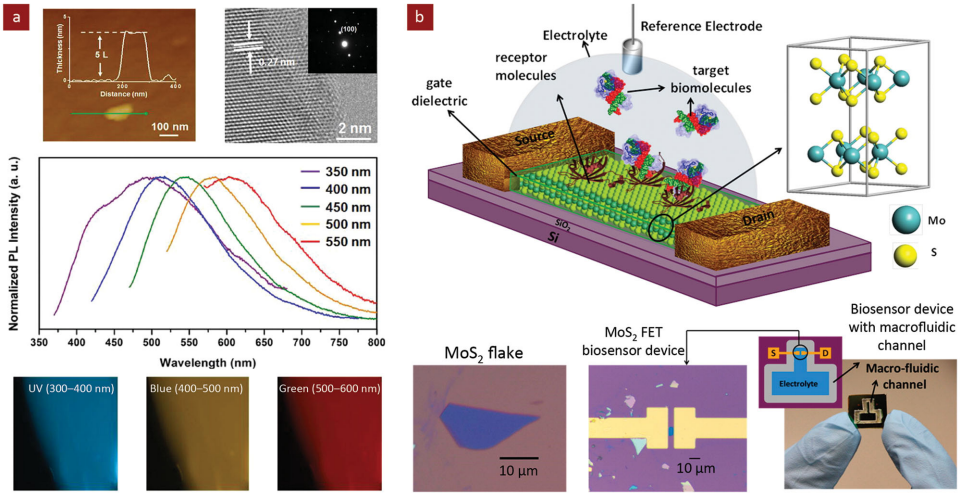

Figure 10. TMDs can be used as biosensors due to their photoluminescence (PL) characteristics. a) AFM and HRTEM images of a typical quasi-2D MoS_2 flake are shown. The plot shows the PL spectra of quasi-2D MoS_2 nanoflakes at different excitation wavelengths. The fluorescent images are of MoS_2 thin films at different excitation wavelengths. b) Schematic of a MoS_2-based FET biosensor device. Reproduced with permission.[76,77] Copyright 2014, American Chemical Society.

including H^+, Li^+, Na^+, and K^+ play vital roles in a wide variety of bioprocesses and have been shown to alter the PL of MoS_2.

In another biosensing study, MoS_2 nanoflakes were used to design a glucose sensor in conjunction with the enzyme glucose oxidase (GOx).[76] The combined MoS_2–GOx system was subjected to different glucose concentrations in vitro under a small voltage (1 V). This experiment showed that the PL of the system increased with increasing glucose concentration up to 50 mM, with a time delay of roughly 60s. This result demonstrated the potential of TMDs for designing nano-sized biosensors. In this experiment the biosensor was saturated at glucose concentrations over 50 mM, which is lower than the expected range of glucose concentrations in vivo. Nevertheless, the use of TMDs for optical biosensing applications is promising and this work could provide insight for the next generation of nano-biosensors.

Finally, MoS_2's potential as a FET has also been utilized in a new approach to FET biosensing (Figure 10b).[77] FETs use an electric field to alter the conductivity of channels in a semiconductor and have attracted considerable attention in biomedical research due to their potential for sensing a wide variety of biological phenomena spanning from protein sensing to pH detection in a fast and inexpensive manner. However, their utility has been impaired due to the limited sensitivity of currently used 3D FETs. Recently, 1D FETs have shown promise for overcoming some of the challenges in 3D FET systems, but their use has been limited by fabrication challenges. Graphene FETs have been attempted but are less sensitive due to their lack of an intrinsic bandgap. Recently, Sarkar et al. developed 2D FETs using MoS_2 nanoparticles.[77] These FET sensors displayed high sensitivity compared to 1D and 3D FETS, as well as facile and low cost fabrication. These FETs were tested as pH and biotin sensors. In the case of the pH sensor, detection was based on the protonation and deprotonation of –OH groups on the dielectric surface. This protonation/deprotonation changes the surface charge of the dielectric surface, which alters the current that can pass through the transistor at a certain voltage. This system was shown to be sensitive across a range from pH 3–9. The biomolecule detection FET utilized a biotin-functionalized surface. A significant decrease in current through the transistor was recorded when streptavidin was introduced into the system due to the negative charge of streptavidin being bound on the FET's dielectric surface. The use of 2D TMDs for this biomedical application is important because their intrinsic properties make them amenable for fabricating cheap, robust, and sensitive FETs. This technology can be expected to lead to significant advances in nanosensor-based diagnostics.[77]

6.2. Tungsten Disulfide (WS_2)

Another 2D TMD nanomaterial being evaluated for biosensing applications is WS_2 nanosheets. WS_2 nanosheets have been shown to have intrinsic peroxidase-like activity that can catalyze the donation of hydrogen from tetramethylbenzidine (TMB) to hydrogen peroxide, a reaction that changes the color of TMB.[78] This feature led to the development of a testing kit for determining glucose levels in blood, in which WS_2 nanosheets were combined with TMB and glucose oxidase to create a sensor that changes color depending on blood glucose concentrations between 5 and 300 μM. Overall, WS_2 nanosheets could lead to the development of highly efficient biosensors by mimicking enzymatic catalysis process.[78]

In addition to their uses in biosensing, the optical properties of TMDs can be harnessed for creating smart drug delivery vehicles. For example, Yong et al. engineered WS_2 sheets as a drug delivery platform for PDT and as a PTT agent (**Figure 11**).[79] PDT uses photosensitizing drugs that can convert O_2 into reactive oxygen species for cancer treatment, whereas PTT uses optically absorptive molecules to efficiently heat cancer cells under near infrared irradiation. TMD nanosheets have attracted research attention for drug delivery applications due to their high surface area, which gives them a protein adsorption capacity comparable to GO. More critically, WS_2 has been shown to be well suited for combining PTT and PDT due to its low toxicity, good water solubility, and high NIR absorption capability. Similar to rGO, TMD nanosheets have a higher extinction coefficient than gold nanorods. They are also easy to purify and are directly dispersible in water. Wang et al. loaded the photosensitizer methylene blue onto bovine serum albumin (BSA)-coated WS_2 for use as a PTT/PDT combined therapy in vitro.[80] The WS_2 were shown to create only a small number of singlet oxygen until activated by 808 nm NIR light, which increased singlet oxygen generation (SOG) by 5 times. The authors proposed that the WS_2 sheets quench singlet oxygen generation when methylene blue is adsorbed, and during NIR activation the methylene blue is released from the nanosheets, effecting the increase in SOG. This mechanism would increase the targetability of PDT therapies, reducing side effects compared to other therapies. Notably, in vivo treatment of induced tumors with the combined PTT and PDT resulted in 20% cell viability, while PTT treatment with WS_2 left 50% of cells viable and PDT only treatment with WS_2 resulted in cell viability exceeding 60%. These results indicated that the combined therapy is more effective than PDT or PTT therapy alone for WS_2, and that combined PTT and PDT therapies may increase the clinical efficacy PDT and PTT cancer therapies.[80–82]

The broad photoresponsive uses of WS_2 are not restrained to cancer treatments; they can be exploited for imaging applications as well. Computed tomography (CT) imaging relies on contrast agents to absorb X-rays, which increases image contrast in the targeted area, and high atomic number elements like tungsten are frequently used as contrast agents due to their high opacity to absorb X-rays. Photoacoustic tomography (PAT), is a newer imaging modality that utilizes non-ionizing radiation and requires NIR absorbance contrast agents. PAT imaging does not penetrate as deeply as CT but offers improved spatial resolution. In a recent paper by Cheng et al., WS_2 nanosheets were successfully adapted as a theranostic device, combining both imaging modalities and PTT functionality. PEG functionalized WS_2 nanosheets were shown to be biocompatible and to passively accumulate in tumor tissue. They were also highly effective for PTT and as CT and PAT bimodal contrast enhancement agents (**Figure 12**). This research suggests that WS_2 nanosheets are a promising material for theranostic treatments, whose combined treatments are expected to improve clinical outcomes and reduce the costs of medical procedures.[82]

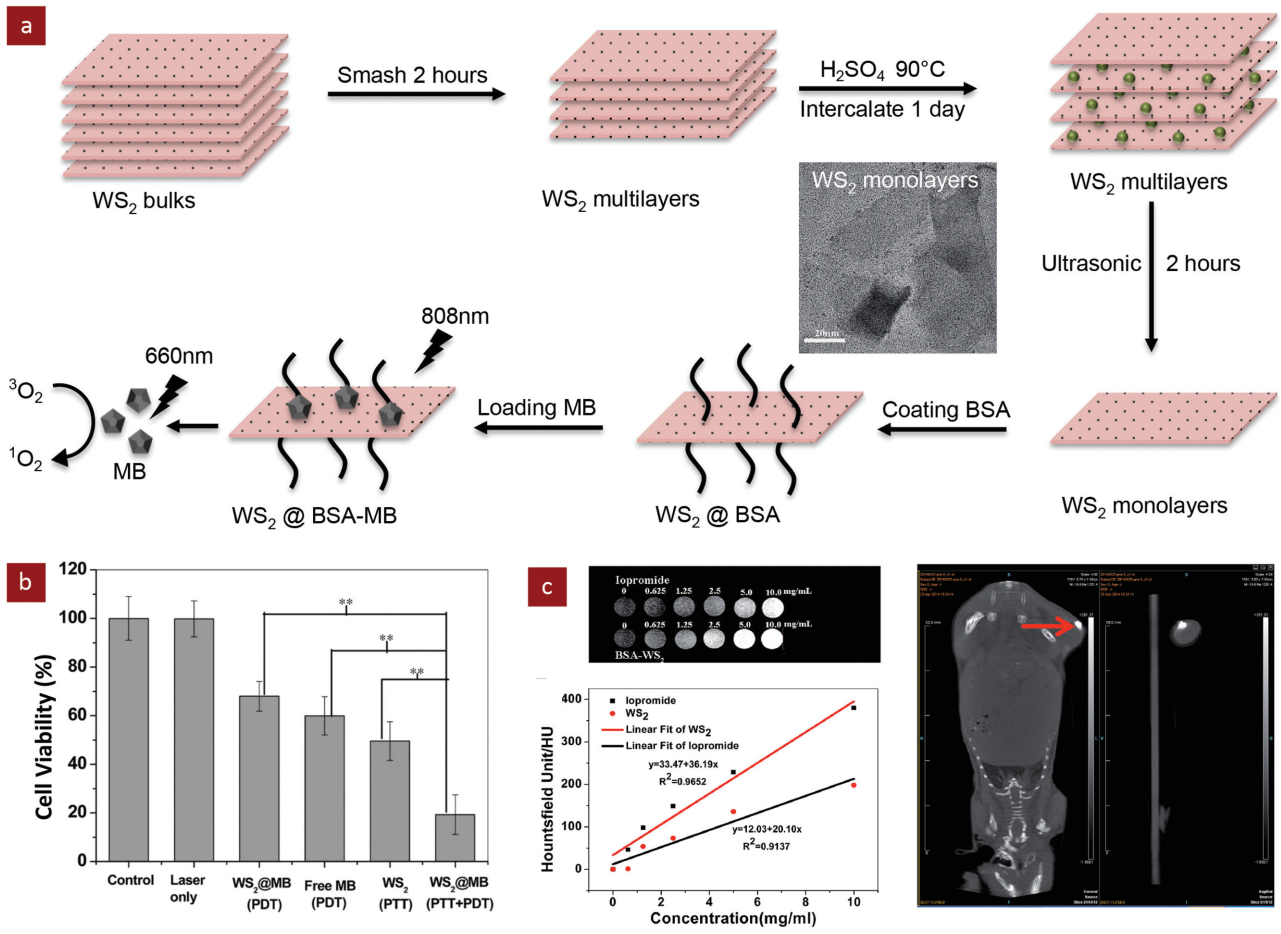

Figure 11. TMDs for drug delivery and phototherapies. a) Schematic showing the synthetic procedure for producing WS_2 nanosheets and their application as a multifunctional photosensitizer delivery system for combined photothermal and photodynamic therapy (i.e., PTT and PDT) of cancer. TEM image of as-synthesized WS_2 nanosheets. b) Effect of different therapeutic approaches (PDT, PTT, and PTT + PDT) on in vitro cytotoxicity. The use of WS_2 nanosheets significantly reduced cancer cell viability, highlighting its efficacy for PTT and PTT+PDT treatments. c) In vitro radiodensity of WS_2 nanosheets at different concentrations. In vivo CT images showing BSA–WS_2 nanosheets. Reproduced with permission.[79] Copyright 2014, The Royal Society of Chemistry.

6.3. Titanium Disulfide (TiS_2)

Recently, a new type of 2D TMD based on titanium (TiS_2) has been explored for PTT and bioimaging applications.[83] Qian et al. used a bottom-up solution-phase approach to synthesize TiS_2 nanosheets that were subsequently modified with PEG to obtain aqueous stable TiS_2–PEG. Preliminary in vitro studies indicated that TiS_2 nanosheets were highly cytocompatible over the short course of these studies. Due to high NIR absorbance by TiS_2–PEG, this material has been used as a theranostic for PTT and as a photoacoustic contrast agent simultaneously. While the results were promising, additional pharmacokinetics data and studies on the long-term dose-dependent toxicology of TiS_2 nanosheets are needed.

6.4. Summary of 2D TMDs for Biomedical Applications

Overall, 2D TMDs are nanomaterials with interesting electronic and optical properties that have primarily been used for nanoelectronics but are seeing increased use in biomedical applications. TMD nanosheets are highly photoluminescent, highly NIR absorbent, have a direct bandgap, and display good mechanical properties, including high rigidity and excellent wear resistance. These properties have made TMD nanosheets an attractive nanomaterial for biosensors, bioimaging, drug delivery, and PTT/PDT treatments. Although TMD research is expanding, the fundamental properties of these materials are not nearly as well understood as some other 2D nanomaterials such as graphene or silicates. Additionally, large-scale fabrication of these materials has proven difficult, and control over a number of specifications like sheet dimensions and quality are still lacking. Nevertheless, TMD nanosheets are promising materials for applications in biomedical engineering and their use should continue to expand as these limitations are addressed in the coming years.

7. Transition Metal Oxides (TMOs) for Biomedical Applications

TMOs are notable among the families of 2D nanomaterials because of their wide diversity of material properties.

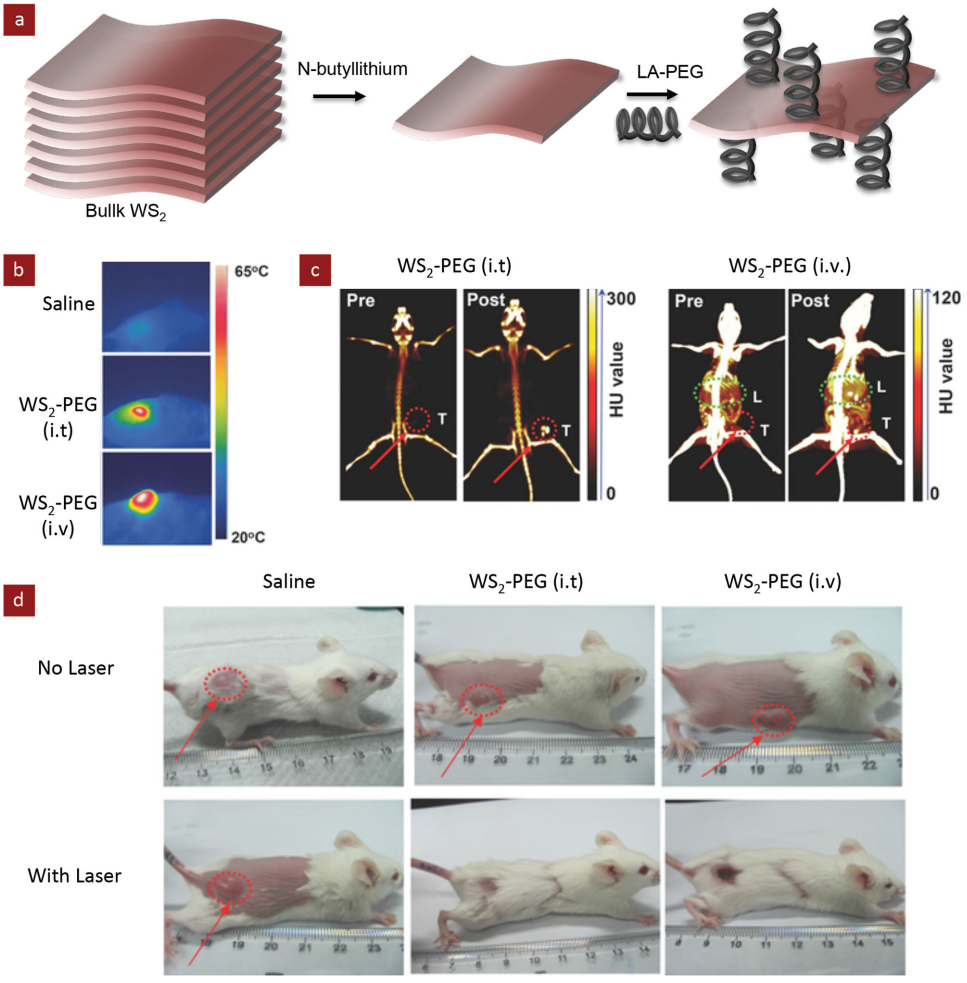

Figure 12. The optical properties of TMDs are being investigated for imaging as well as PTT treatment. a) WS_2 can be exfoliated and functionalized with PEG to enhance stability in salt solutions. b) IR imaging results showing the in vivo heating of WS_2–PEG nanoparticles via infrared laser irradiation. c) CT imaging of tumor-bearing mice after intratumoral (IT) or intravenous (IV) WS_2–PEG administration demonstrating enhanced contrast, particularly in tumors and the liver. d) In vivo tumor reduction with WS_2–PEG PTT. Reproduced with permission.[79,82] 2014, John Wiley & Sons, Inc.

Nanomaterials in this family typically have wide bandgaps, giving them unique photochemical and electric properties. Other nanomaterials in this family have been shown to exhibit ferromagnetic and redox properties, and high thermal resistance. TMO nanoparticles have a relatively long history of study into their material properties, but this research has focused almost exclusively on 0D, 1D, and 3D nanostructures due to the relative difficulty of fabricating 2D TMOs.[10,63,84] In the past few years, delamination and bottom up synthesis of TMOs have made their study more practical. The members of this family that have been successfully delaminated into nanosheets include titanium dioxide (TiO_2), manganese dioxide (MnO_2), zinc oxide (ZnO), cobalt (II, III) oxide (Co_3O_4), tungsten trioxide (WO_3), and iron (II, III) oxide (Fe_3O), among others. Materials in this family commonly feature divalent cation interlayers, which keep the sheets in stacks. These interlayers can be broken up through cation exchange to substitute bulkier cations into this interlayer, pushing the sheets farther apart and weakening electrostatic interactions.[84,85] Due to the novelty of the 2D forms of these materials, relatively little biomedical research has been completed on them compared to TMDs, and particularly graphene. Most of the research that has been published has focused on two of the better studied materials: MnO_2 and TiO_2.[63,86] In this section, we will highlight some of the promising research areas on TMOs for biomedical applications.

7.1. Manganese Dioxide (MnO_2)

Manganese dioxide (MnO_2) nanosheets are composed of metal oxide octahedral unit monolayers, alternating with interlayer cations. MnO_2 exhibits cation exchange properties, is semimetallic, and electrochromic. MnO_2 is particularly noteworthy for its facile redox activity relative to other TMOs. MnO_2 nanosheets have been used for biosensing, imaging, and drug delivery applications. For example, a recent paper by Chen et al. used MnO_2 nanosheets for a theranostic treatment in which the material acted as a contrast agent for magnetic resonance imaging (MRI) but could also steadily break

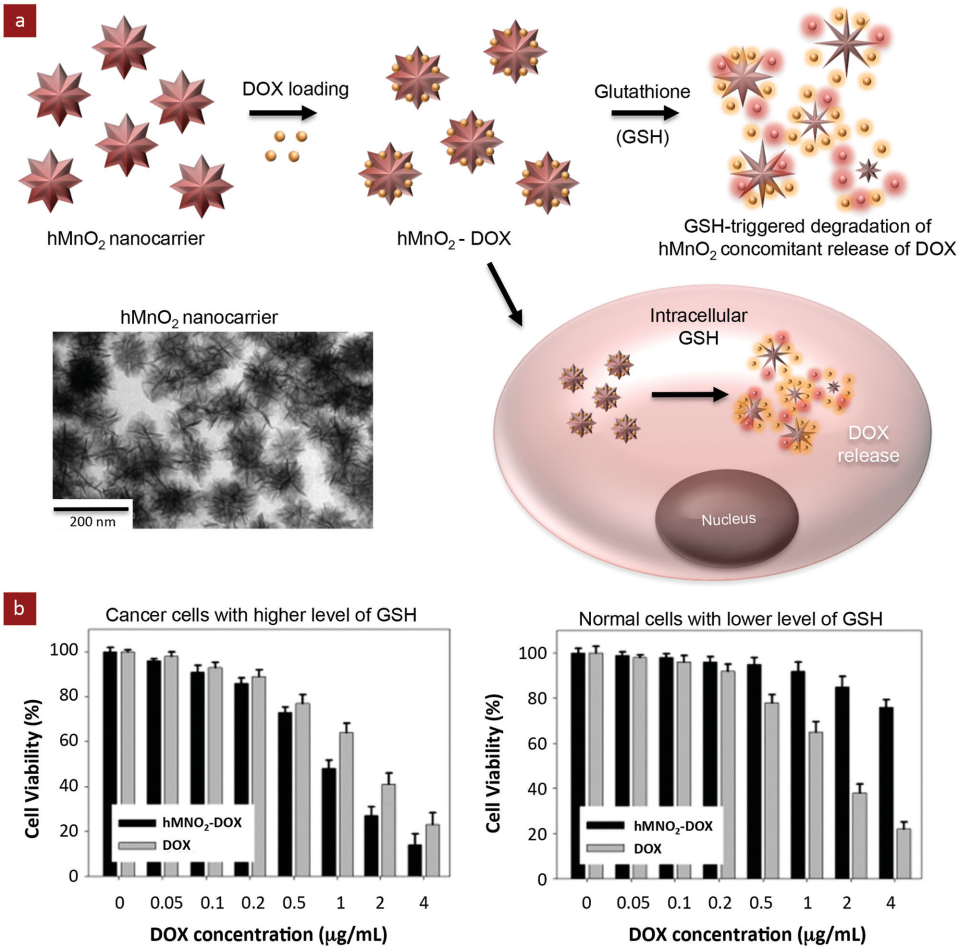

Figure 13. Application of TMOs for drug delivery. a) TMO nanocarriers, such as MnO_2 nanoplatelets, can efficiently transport drugs to tumor cells. TEM images of MnO_2 nanocarriers are shown. MnO_2 particles are internalized inside the cell body and quickly dissolve in the presence of intracellular glutathione to release DOX. b) Delivery of DOX using MnO_2 nanocarriers was effective compared to free DOX. Moreover, MnO_2 nanocarriers were specific to tumor cells (left) over normal cells (right). Reproduced with permission.[87] 2014, John Wiley & Sons, Inc.

apart under the mildly acidic conditions found inside tumors to deliver therapeutics (**Figure 13**).[87] MnO_2 nanosheets were modified with PEG to improve their stability under physiological conditions and loaded with doxorubicin (Dox) for targeted drug release. Under in vivo conditions, these nanosheets were shown to increase MRI signal intensity within tumors. This result was due to the release of Mn(II) particles within the tumor upon breakup of the nanosheet. Additionally the MnO_2–Dox–PEG system resulted in 59.6% cell death in 48 hours, compared to 26.5% cell death from free Dox. This combined dual-functionality system represents an important tool in the development of cancer therapies by combining intelligent design of drug delivery and tumor imaging systems using 2D nanomaterials.[88]

MnO_2 nanosheets have also recently been developed for biosensing applications, including as fluorescence resonance energy transfer (FRET) biosensors.[89,90] FRET biosensing is a staple technique in biosensing research and is used to detect ion concentrations, pH, proteins, metabolites, cancer cells, and DNA sequences. MnO_2 nanosheets have received significant attention for these applications due to their utility in energy storage technologies, including supercapacitors and lithium batteries. MnO_2 nanosheets are known to be effective energy absorbers, and due to their high surface area and light absorption they have been explored for use as FRET biosensors. Yuan et al. used MnO_2 nanosheets to develop a label-free platform for homogenous FRET biosensing. MnO_2 nanosheets were combined with aptamers conjugated to fluorophores to create FRET biosensors for ochratoxin A and cathepsin D. Because of the water solubility of MnO2 as well as its broad, intense light absorption, which is due to its lattice structure and makes MnO_2 adaptable to a wide range of fluorophores in different spectral regions, these probes have been tested under a range of physiological conditions in order to ensure their specificity. These tests indicated that MnO_2 nanosheets are a favorable nanoplatform for homogenous biosensing, easy to fabricate, amenable to a wide range of conditions, highly specific, and robust.[90]

In another biosensing application study, Deng et al. combined MnO_2 nanosheets with lanthanide-doped upconverting nanoparticles for rapid, selective detection of glutathione in aqueous solutions and living cells.[91] In this approach, they

synthesized MnO_2 nanosheets on nanoparticle surfaces, and the MnO_2 nanosheets acted as a quencher for upconverted luminescence. The addition of glutathione reduces MnO_2 into Mn^{2+}. Thus by monitoring the glutathione concentration intracellularly, a platform for targeted drug and gene delivery can be designed.

7.2. Titanium Dioxide (TiO_2)

Another TMO that has been used for biomedical applications is TiO_2 nanosheets. Although TiO_2 has a similar structure to MnO_2, it has significantly different properties. TiO_2 is semiconductive with a wide bandgap, giving it good optical absorbance at UV wavelengths, and has a high-*k* dielectric constant. These features have led to TiO_2 being evaluated for PDT, cell imaging, biosensing, and drug delivery applications. TiO_2 is used as shorthand for titanium oxide in this section because it approximates the formula of titanium oxides. However it is not necessarily the precise composition of titanium oxide.[14,85,92] It is well known that TiO_2 nanoparticles produce oxidative radicals upon exposure to UV light (<385 nm). These radicals have been shown to be capable of killing a wide variety of cells and have demonstrated in vivo efficacy. Nitrogen-doped TiO_2 nanosheets showed improved visible light absorbance, resulting in higher radical production.[93] This shift from UV to visible light makes PDT with TiO_2 nanoparticles more practical for clinical application as a noninvasive cancer treatment. Additionally, Elvira et al. incorporated specificity into TiO_2 by immobilizing monoclonal antibodies for neural stem cells onto the nanoparticles.[94] The antibody-associated nanoparticles were shown to accumulate on the target cells, which resulted in highly selective PDT. This approach has the potential to lead to the development of highly specific cancer therapies and expand the therapeutic options available to patients. In another application, TiO_2 nanosheets were combined with acrylamide to fabricate nanocomposite hydrogels that mimicked some of the physical and chemical properties of articular cartilage.[95] This technique relied on the use of a strong magnetic field to coaxially align the TiO_2 nanosheets before polymerization. This new nanocomposite design has many potential applications in biomedical engineering as a new paradigm in nanocomposites.

7.3. Summary of 2D TMOs for Biomedical Applications

Overall, TMOs are being investigated for bioimaging, biosensing, PDT, and drug delivery applications. These applications of 2D TMOs stem from their redox activity, cation exchange capabilities, and varied electrical and optical characteristics. TiO_2 nanosheets have frequently been used for their photocatalytic and dielectric properties, while MnO_2 nanosheets have been more often used for their semi-metallic and redox properties. TMO nanosheets, like many other 2D nanomaterials, remain insufficiently evaluated for biocompatibility. In the future, new biomedical research in this area should further leverage the optical and electronic properties of these materials.

8. Other Types of 2D Nanomaterials

Some 2D nanomaterials do not fit neatly into any of the categories described above. These materials form single layer nanosheets like graphene but do not consist of simple repeating patterns of carbon atoms. Graphitic carbon nitride, for example, alternates carbon and nitrogen heteroatoms, forming a strong, regular structure with semiconducting and catalytic properties. Similarly, hexagonal boron nitride forms a hexagonal monolayer of alternating boron and nitrogen atoms. Other materials include silicene and germanene, which are group IV elements that share many atomic properties with carbon. These elements have long been speculated to be able to form their own 2D allotropes, but these allotropes were not synthesized until this decade. In this final section, these unique materials are examined individually in the context of biomedical engineering.

8.1. Graphitic Carbon Nitride (C_3N_4)

2D, or graphitic carbon nitride (C_3N_4) is a relatively new material, having only been synthesized in the last couple of years.[96,97] Ultrathin graphitic C_3N_4 (g-C_3N_4) nanosheets were produced for the first time in 2012, and have been found to exhibit high intrinsic photoabsorption and photoresponsiveness, semiconductive properties, high stability under physiological conditions, and good in vitro biocompatibility.[75,96–99] These properties lend the material to applications as an imaging agent, drug delivery vehicle, and in biosensing. C_3N_4 nanosheets may improve the current state of the art in bioimaging by providing a nontoxic alternative-imaging agent. C_3N_4 sheets have been demonstrated to be able to bind to cell nuclei, enabling facile intracellular fluorescence imaging due to their high PL quantum yields.[98] Studies have also indicated that these nanosheets may be especially useful for phototherapies after they have been endocytosed into the cell. For example, in Lin et al., C_3N_4 nanosheets were demonstrated to enhance confocal fluorescence imaging without damaging living HeLa cells (**Figure 14**).[99,100] C_3N_4 nanosheets have also been shown to have a high capacity for drug loading, being able to bind up to 18 200 mg g^{-1} of doxorubicin.[99] These nanocarrier sheets were shown to have significant in vitro anti-cancer activity, though not as much as free doxorubicin. Doxorubicin release was achieved via a pH-responsive mechanism: increased hydrophilicity of doxorubicin at lower pHs caused it to dissociate from the nanocarrier. In another recent trial, ultrathin C_3N_4–Fe_3O_4 nanocomposites were shown to have high photocatalytic efficiency.[96] Carbon nitride nanosheets have also been shown to be an effective biosensor due to their semiconductive properties, fluorescence, and high stability under a wide variety of conditions. Ma et al. showed that protonated C_3N_4 nanosheets can be used to directly monitor heparin concentration.[101] A broad linear heparin concentration range from 0.05–5 μg mL^{-1}, which is clinically relevant, can be quantified with excellent sensitivity and specificity. C_3N_4 has also been demonstrated as an effective biosensor for glucose and in combination with GO as a simultaneous biosensor for ascorbic acid, dopamine, and uric acid.[102] Although C_3N_4 nanosheets are relatively new 2D nanomaterials, they are clearly useful for biomedical

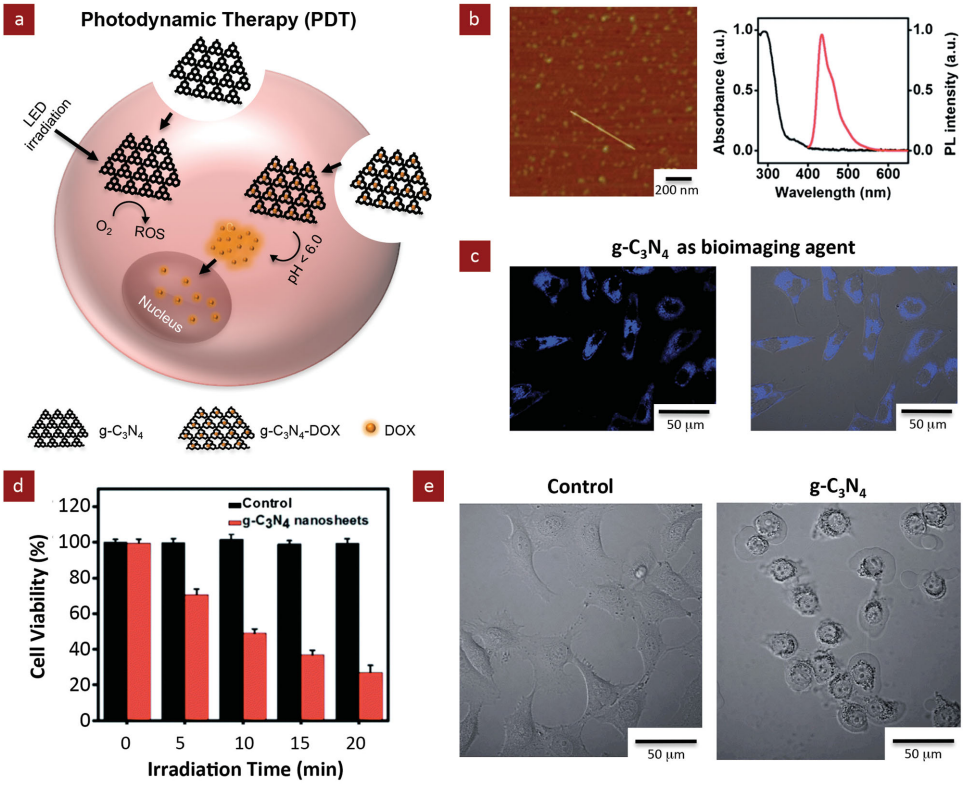

Figure 14. Application of g-C_3N_4 for intracellular imaging, PTT and PDT. a) g-C_3N_4 nanosheets can be used to deliver therapeutic cargo and for PDT due to their ability to absorb visible light. b) AFM images and absorption spectra of g-C_3N_4 nanosheets. c) g-C_3N_4 nanosheets can be used as bioimaging agents. d) Cell viability results demonstrating the ability of g-C_3N_4 nanosheets to be used for PDT therapy. e) Cell morphology before and after PDT with g-C_3N_4. Reproduced with permission.[99] Copyright 2014, The Royal Society of Chemistry.

applications and could also be used to engineer efficient medical devices.

8.2. Hexagonal Boron Nitride (hBN)

Similar to graphitic carbon nitride, hexagonal boron nitride (hBN) nanosheets are also monolayers with alternating nitrogen moieties.[6,103–105] However, hBN alternates nitrogen with boron atoms, which are less electronegative than carbon. The stronger polarity of the nitrogen-boron bond affects many of its properties, including its UV luminescence and wide bandgap semiconductivity. Because of these characteristics as well as its chemical and thermal stability and wide availability, hBN has garnered significant attention for use in electronics applications.[103] Importantly, hBN monolayers make the synthesis of hybrid nanostructures of graphene and hBN with tunable bandgaps possible, which is a widely sought after goal in electronics research.[106] However, the unique properties of hBN also make it a promising material for a wide variety of biomedical applications that have just begun to be explored. hBN has a number of favorable biological properties, including cytocompatibility and a demonstrated propensity for being endocytosed into cells. Like all 2D nanomaterials, the biocompatibility of hBN has yet to be definitively established, but preliminary studies have shown that hBN may have better biocompatibility than graphene. This research has led to exploration of hBN as a more biocompatible alternative to graphene in drug delivery, imaging, and biosensing applications.[6,104,105]

Like graphene, pure hBN is not suitable for drug delivery due to its poor solubility in physiological solutions.[107] However, also like graphene, hBN can be functionalized through hydroxylation to increase its water solubility. Recently, a new synthesis method involving thermal substitution of boric acid with C_3N_4 has been demonstrated to produce highly hydroxylated hBNs that have high water solubility (2.0 mg mL^{-1}) and low cytotoxicity in vitro (**Figure 15**).[107] The behavior of hydroxylated hBN nanosheets has been compared to that of graphene oxide. Weng et al. showed that hydroxylated hBNs were capable of adsorbing up to 300% of their weight in doxorubicin.[107] Drug-loaded hydroxylated hBNs also exhibited pH dependent release kinetics, with acidic pHs freeing doxorubicin in higher amounts and at higher rates. While drug-free hydroxylated hBN was highly cytocompatible, the doxorubicin-loaded nanosheets reduced cancer cell viability to 18–21%. The authors suggested that this increase in toxicity over free DOX was the result of the nanosheets being endocytosed and releasing their payload in the acidic lysosomes. The combination of high biocompatibility and drug loading, and improved antitumor effectiveness makes hydroxylated hBN a possible clinical drug delivery vehicle that could improve the effectiveness of chemotherapeutics while also reducing side effects.

The endocytosis and biocompatibility of hBN nanosheets mentioned above also make them an attractive material for

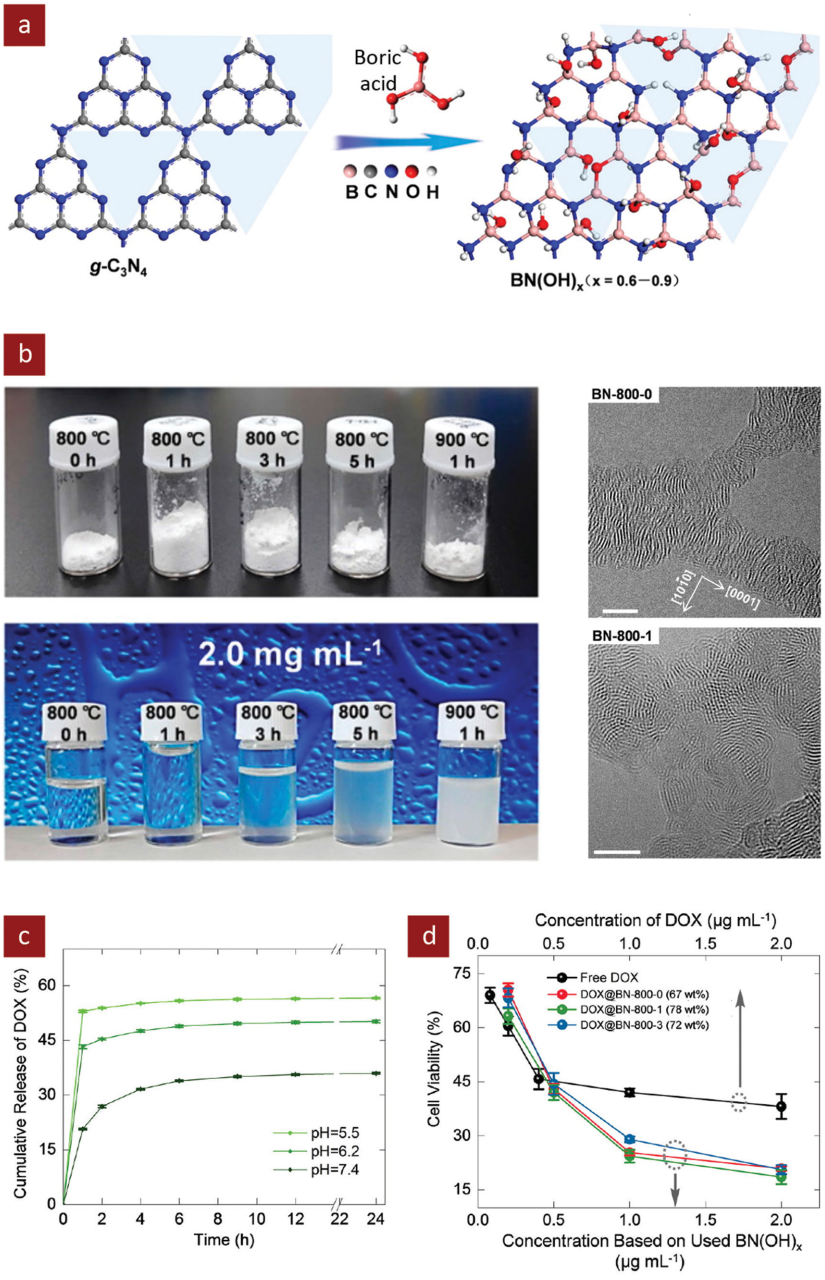

Figure 15. hBN nanosheets for drug delivery applications. a) Surface modification of g-C_3N_4 was performed with boric acid treatment to obtain b) water-soluble hBN. HRTEM images of hBN showing the sheet network. c) The release of DOX from DOX@BN networks was affected by pH conditions. d) Cell viability results showing the effect of DOX release from the BN network on human prostate cancer cells. Reproduced with permission.[107] Copyright 2014, American Chemical Society.

live cell imaging. Peng et al. functionalized hBNs with graphene quantum dots (GQDs), taking advantage of their stability and endocytosis to create a new intracellular fluorescent marker.[108] Green fluorescent GQDs were incorporated onto hBN nanosheets and exposed to HeLa cell cultures. The results demonstrated strong fluorescence, stability, good solubility, and low cytotoxicity, as well as the ability to efficiently penetrate cells. This technology has the potential to expand the capabilities of live cell imaging technologies by increasing the stability of intracellular fluorescent markers.

One area of biomedical engineering where hBN nanosheets are noticeably absent is tissue engineering. Given their shape, strength, and biocompatibility, hBN nanosheets have excellent potential for use in tissue engineering applications. The use of hBN nanosheets specifically in hydrogel nanocomposites has yet to be explored, though hBN nanotubes have recently demonstrated osteoinductive effects on MSCs.[109] However, a range of nanocomposites have been fabricated from hBN by combining it with different polymers.[110] For example, a nanocomposite of hBN with gelatin has been fabricated[105] and, due to the similarity of gelatin with the native ECM of tissues, these nanocomposites can be explored for tissue engineering and stem cell applications. It is expected that this new type of 2D nanomaterial can be used as a reinforcing or bioactive agent within a hydrogel matrix to modulate the differentiation of stem cells.

Overall, hBN has shown compelling mechanical and chemical properties that suggest many potential uses in biomedicine. Thus far, however, the exploration of hBN's applications has been hampered by synthetic difficulties. The chemistry of boron and nitrogen is not nearly as well explored as that of carbon, which limits researchers' ability to manipulate and functionalize hBN. Additionally, synthesis and dispersion difficulties continue to slow research progress. Once these problems are overcome, hBN's role in biomedical engineering can be expected to expand significantly.

8.3. Silicene and Germanene

Recently, new 2D allotropes known as silicene and germanene have been reported.[111] The basic physical properties of silicene, synthesized in 2012, and germanene, synthesized in 2014, are only just beginning to be explored. Silicene and germanene have a similar electrical conductance to graphene. However, these 2D nanosheets also have unique and useful properties, such as high flexibility and compressibility, as well as strain dependent increases in thermal and electrical conductivity. Recently, it was reported that both 2D silicene and germanene have high structural stability, high phonon scattering ability, and high energy electrical transport properties.[112] These interesting properties coupled with their ability to integrate with electronic devices can provide multiple opportunities in the area of bioelectronics. Until facile synthetic

techniques are developed for these materials, however, these materials will remain unfortunately rare and scarcely explored. More information on the calculated properties of silicene and germanene can be found in these reviews.[6,7,113]

9. Emerging Trends and Future Outlook

The biomedical applications of 2D nanomaterials are rapidly expanding and promising improvements in areas such as bioimaging, drug delivery, biosensors, tissue engineering, photothermal therapy, photodynamic therapy, and hemostatic wound dressings have been demonstrated. The complex relationships between material properties, structure, shape and defects have emerged as a field that is ripe for development across the scientific community. With our recently acquired knowledge of the rules of nature that govern the atomic-, nano-, micro-, and macro- scales, we are well positioned to unravel and control the complexity that determines functionality of these newly developed 2D materials.

In the future, the field of 2D nanomaterials will likely include a wider selection of nanomaterials that includes novel materials as well as a better understanding of the physical, chemical and biological characteristics of different types of 2D nanomaterials that govern their utility in biomedical and biotechnological applications. New 2D nanomaterials that have only just been synthesized, like silicene and germanene, have yet to be fully characterized, and are a long way from translational applications. However, these and other undiscovered materials may have unprecedented properties that could hold the keys to new biomedical research breakthroughs.

One particularly important emerging trend in nanomaterials is the development of a new generation of intelligent structures that are multifunctional, adaptive, programmable, and biocompatible. By combining rational design of 2D nanomaterials with computational modeling and detailed physical, chemical and structural characterizations, it is possible to engineer designer materials. Specifically, there is immense interest in engineering nanocomposites loaded with nanomaterials with tunable mechanical, structural, chemical, and biological properties through precise control of size, shape, and composition of the 2D nanoscale building blocks. With proper orientation and assembly, 2D nanomaterials could potentially lead to 3D mesostructures with unrivaled properties for a host of technologies. While the importance of mesostructured materials is well appreciated in the broader materials science community, the implications for biomaterials can perhaps best be appreciated by considering that, while nature uses a variety of nanoscale building blocks, the key properties of cells, tissues, and organisms rely on the proper assembly of these building blocks into larger mesoscale structures. Mesoscale particles comprising drug-loaded nanoparticles have shown promising results for multi-stage controlled delivery of therapeutic agents.[114] However, thinking more broadly, a more complete understanding of how nanomaterials can be assembled into mesostructured materials and devices could lead to exciting advances throughout biomedical engineering. Thus, it is our expectation that the introduction of 2D nanomaterials to the biomedical community represents a paradigm-shift in applying fundamental materials science knowledge to biomedical engineering that will enable engineering of the next generation of medical devices.

As we move from basic science towards translational research, an increase in novel combinations of nanomaterials as well as innovative processing strategies and applications will emerge. Nanocomposites containing multiple dimensions of nanomaterials such as 0D, 1D, 2D, and 3D nanostructures have recently been reported to exhibit synergistic properties combinations. Some of these nanocomposites are highlighted in carbon-based 2D nanomaterials. Combinations of carbon nanotubes and 2D nanomaterials like graphene, clays, LDHs, or TMDs are beginning to attract attention as a way to obtain synergistic property combinations. For example, a nanocomposite comprising polypropylene (PP) loaded with GO and CNTs exhibited increased tensile strength, elastic modulus, and electrical conductivity, among other properties.[115] In the future we anticipate that many material combinations will be explored for biomedical applications.

As new types of 2D nanomaterials are discovered and characterized, their interactions with biological entities need to be investigated in detail. Specifically, it is important to understand the effects of 2D nanomaterial size, shape, chemical composition and surface characteristics on protein adsorption, cellular internalization, and binding to sub-cellular components. Recent literature indicates that 2D nanomaterials can be exploited to control stem cell fate. For example, graphene,[21] silicate clay,[55] and hBN[109] have all been shown to induce the osteogenic differentiation of stem cells in the absence of any other osteogenic agents. At the atomic-level there is nothing common between these materials except their 2D shapes. Thus, from our observations, there is a strong indication that nanoparticle shape may play a major role in directing cellular processes including differentiation, proliferation, adhesion and migration. As the field advances, it will be critical to understand the interactions of 2D nanomaterials with cellular components such as structural proteins, genetic materials, and metabolites, to understand how these nanomaterials control or affect various signaling pathways. This knowledge will broaden our understanding of how nanomaterial shape can be used to control specific biological process.

One of the primary challenges facing the biomedical application of 2D nanomaterials is biocompatibility. Because 2D nanomaterials is a nascent field, only a few types of 2D nanomaterials have been evaluated for biocompatibility. Moreover, the cyto- and biocompatibility of 2D nanomaterials are poorly understood due to several confounding factors such as size and shape, which are difficult to control with current synthesis methods. This lack of control over the structure of 2D nanomaterials makes the systematic evaluation of their biological interactions challenging. Thus, there is an immediate need to address this problem so that efforts can be focused towards identifying a library of 2D nanomaterials that are appropriate for various biomedical applications. Additionally, most biocompatibility evaluations to date have been superficial, and the long-term effects and the fate of 2D nanomaterials inside the body are not known. Long-term in vivo evaluations are necessary before the promising benefits demonstrated by many 2D nanomaterials can be translated into clinical use.

10. Conclusion

2D nanomaterials are ultrathin nanomaterials with a high surface-to-volume ratio as well as anisotropic physical and chemical properties compared to 3D nanomaterials. Because one of the dimensions of 2D nanomaterials is only a few atomic layers thick, they interact with biological moieties in a unique way, which has raised exciting questions about their interactions with proteins, cells and sub-cellular components. Once the biocompatibility of these materials is confirmed, their exceptional properties have the potential to be translated from bench to bedside, which could lead to transformative advances in biomedical science and clinical outcomes. Here, we attempted to review the most promising technologies in 2D nanomaterials, which only represents a fraction of this exciting and rapidly expanding field. Graphene, GO, and rGO remain the focal points of research in the biomedical field, and recently novel 2D nanomaterials such as clay, LDH, TMDs, and TMOs have attracted interest and are being evaluated. The biocompatibility, bioactivity, uniform particle size, and permanent surface charges of LDHs and clays are being exploited primarily for drug delivery, tissue engineering, hemostatic wound dressings and biosensing. Meanwhile, the relatively underutilized TMOs and TMDs are finding utility in bioimaging, biosensing, drug delivery, photothermal therapy, photodynamic therapy, and novel cancer treatments. Recently investigated materials like hBN and C_3N_4 nanosheets have shown promise for a variety of applications due to their similarity to graphene, and these materials are likely to be evaluated extensively once their synthetic difficulties are overcome. Some of the emerging trends in 2D nanomaterials are the development of intelligent structures that are multifunctional, adaptive, programmable, and biocompatible. These unique structures can be obtained by designing multi-component systems as well as innovative processing strategies. Overall, unprecedented challenges and opportunities exist in developing 2D nanomaterials for next-generation technologies for basic cell biology, medical diagnostics, regenerative medicine, drug and gene delivery, stem cell engineering, cancer therapy, biosensing, and bioelectronics.

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