SOAR

STATE-OF-THE-ART REPORT (SOAR)
AUGUST 2022

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NANOTECHNOLOGY APPLICATIONS FOR COMBAT CASUALTY CARE

By Gregory P. Nichols, Loren Shelby, and Deanna C. Milonas
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GREGORY P. NICHOLS, LOREN SHELBY, AND DEANNA C. MILONAS

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This state-of-the-art report focuses on advances in combat casualty care (CCC) treatments, tools, and techniques enabled by the application of nanotechnology. Recent research and development at the nanoscale have yielded innovations in hemorrhage control and fluid resuscitation, wound management and infection, bone regeneration and engineering, neurotrauma and pain control, and advanced medical monitoring and diagnostics. For each of these areas, we review current trends in applied and translational research, commercially available products (including those funded by the U.S. Department of Defense [DoD]), existing research gaps, and technical- and policy-related implementation challenges. Overall, we detail the ways in which nanotechnology is generating significant improvements to DoD's CCC capabilities and helping the military's medical mission anticipate and prepare for the future battlefield while continuing to improve casualty outcomes.

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ABSTRACT

This state-of-the-art report focuses on advances in combat casualty care (CCC) treatments, tools, and techniques enabled by the application of nanotechnology. Recent research and development at the nanoscale have yielded innovations in hemorrhage control and fluid resuscitation, wound management and infection, bone regeneration and engineering, neurotrauma and pain control, and advanced medical monitoring and diagnostics. For each of these areas, we review current trends in applied and translational research, commercially available products (including those funded by the U.S. Department of Defense [DoD]), existing research gaps, and technical- and policy-related implementation challenges. Overall, we detail the ways in which nanotechnology is generating significant improvements to DoD's CCC capabilities and helping the military's medical mission anticipate and prepare for the future battlefield while continuing to improve casualty outcomes.

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EXECUTIVE SUMMARY

Combat Casualty Care (CCC) aims to provide an injured Warfighter with immediate trauma management, a path that runs from the initial point of injury all the way through the transition to downstream facility-based care. As U.S. national defense strategy pivots away from counterterrorism and toward "great power competition" and as the services modernize and restructure to prepare for large-scale combat operations, the face of CCC is also primed to change. For example, U.S. Army leaders expect future conflicts will bring an uptick in the incidence of burn injury, see higher overall casualty rates, and place new demands on medical logistics. Central to this effort is evaluating the application of nanotechnology to CCC practices and products. Nanotechnology is the manipulation of matter at the atomic scale, with at least one dimension less than 100 nm (or less than one billionth of a meter) and has been a heavily supported research and development (R&D) area by the U.S. Department of Defense (DoD) for more than 20 years.

To produce this report, the Homeland Defense and Security Information Analysis Center (HDIAC) reviewed a broad array of scientific and engineering literature, including peer-reviewed publications, government reports, project reports from DoD-funded efforts, commercial pamphlets, and other sources of relevant "gray" literature. HDIAC also interviewed a dozen subject matter experts in medicine, engineering, nanoscience, and other relevant fields, as well as project managers and other DoD leaders who oversee research funding in nanotechnology or CCC. We focused on products that are commercially available, currently used in the field, or in late-stage commercialization (nearing clinical trials, in clinical trials, or at a high technology readiness level). Most emerging trends convey research that is beyond basic and fundamental stages.

The core content of this report covers five interrelated areas and begins with Section 2, "Hemorrhage Control/Fluid Resuscitation." Multiple products exist now to provide synthetic blood substitutes and fluid replacement for CCC. This section reviews some of the most promising new products (SynthoPlate, ErythroMer, and $NanO_2$) and discusses emerging nanotechnology research in whole blood. Overall, all roads of relevant R&D in this area lead ultimately to the primary goal of creating a viable whole blood alternative.

Section 3, "Wound Management and Infection," offers an overview of the crowded market space that wound care currently inhabits. The primary focus is on dressings and bandages made from various types and configurations of nanomaterials. A glimpse of available technologies for wound closure, infection control, and burn care is also provided. Key materials in this space are nanoscale chitosan and silver nanoparticles (NPs). Most nanoenabled wound care products are constructed from electrospun fibers, nanogels, and nanoemulsions.

While generally more advanced care than what is provided on the battlefield, tissue regeneration has become an important part of CCC research, mainly because it can be viewed as an extension of wound care. Section 4, "Tissue Regeneration and Engineering," covers the specific case of bone tissue engineering (BTE), as this represents the most advanced tissue regeneration effort. Other types of tissue engineering are covered throughout other

EXECUTIVE SUMMARY, *continued*

sections. This section discusses some of the most promising current and emerging developments in BTE, including the NuCress scaffold from NuShores Biosciences, recent work on incorporating mesenchymal stem cells into BTE, and three-dimensional (3-D) printing used in BTE.

Section 5, "Neurotrauma and Pain Control," focuses on brain and nervous system injuries and is an extremely important part of modern CCC. This section provides a broad sweep of areas, including neuroprotection, nanosensors, and advanced rapid diagnostic tools to detect and mitigate brain injury (such as the NanoDx platform), and nanopharmaceutical research for addressing pain control and traumatic brain injury using intranasal delivery methods.

The core content of the report ends with Section 6, "Advanced Medical Monitoring and Diagnostics." Uses of nanotechnology to enhance medical monitoring during prolonged field care and en route care are discussed as well as emerging areas of R&D for real-time biosensing, theragnostics, and converging technologies (e.g., internet of things and 3-D printing). Research in the field demonstrates a trend toward using advanced, nanoenabled tools to move the point of care to the individual and extend the capabilities of field medical personnel.

Overall, nanotechnology in CCC illustrates how important miniaturization has become in biomedical applications and how dramatic improvements and new and novel technologies have the potential to disrupt innovation in remote medical care. This research revealed some clear trends and themes that weave throughout the entire practice of nanotechnology in CCC, including the ubiquitous use of chitosan for nearly all types of medical applications; the expansion of lipid NPs as carriers for all types of therapeutics; nanotechnology contributing to the concept of convergence; and the incorporation of nanomaterials into the broader category of advanced materials. One of the key themes is a paradigm shift toward defining solutions and accepting what may come. Although much of this new research still includes nanotechnology approaches because of the level of complexity needed to solve the problem, especially from a medical perspective, it is often improved at the nanoscale.

Finally, this report should serve as a beginning point for further R&D and critical evaluation of how CCC can continue to improve. Nanotechnology applications in other fields such as oncology, neurology, diabetes care, and orthopedics have shown successes that could be adapted for CCC. Additionally, other agencies with relevant missions to remote and emergency care (U.S. Departments of Energy, Health and Human Services, and Homeland Security, and the National Aeronautics and Space Administration) may offer advanced technology solutions in spaces that on the surface may not seem relevant but end up being beneficial.

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SECTION 01 INTRODUCTION

1.1 OVERVIEW

To enable a medically ready force, the U.S. Department of Defense (DoD) operates a vast network of prevention, protection, and treatment support for Warfighters and other DoD personnel. In fiscal year 2021, appropriations for the Defense Health Program totaled more than $37 billion, a full 10% of which went to efforts in research, development, testing, and evaluation (RDT&E) to innovate across the spectrum of medical science [1]. To provide DoD medical personnel with tools and techniques that go beyond traditional first responder equipment, the DoD also operates research groups like the Joint Program Committee-6/Combat Casualty Care Research Program, which develops solutions for treating combat-related trauma to optimize survival and recovery.

While joint medical capabilities seek to achieve a 0% preventable death rate among all DoD personnel [2], chief among its medical missions is providing combat casualty care (CCC; sometimes referred to as tactical combat casualty care). The aim of CCC is to provide an injured Warfighter with immediate trauma management from the point of injury (POI) through the transition to facility-based care [3]. U.S. involvement in the wars in Afghanistan and Iraq significantly advanced CCC medical capabilities over the past two decades. For example, between 2005 and 2013, the fatality rate for U.S. service members injured in Afghanistan decreased by 50%—even as injury severity increased [4]. By one estimate, U.S. military medicine has achieved the "best casualty outcomes in the history of modern warfare" [5].

As U.S. national defense strategy pivots away from counterterrorism and toward "great power competition" and the services modernize and restructure to prepare for large-scale combat operations (LSCO), the face of CCC is also primed to change. Compared to recent U.S. operations in the Middle East, our adversaries in LSCO will present U.S. forces with a wider array of threats (including artillery and high-precision missile fires), contest U.S. air operations (including medevac), and require U.S. forces to manage CCC in new and rapidly changing battlefields. U.S. Army leaders expect future conflicts will bring an uptick in the incidence of burn injury, see higher overall casualty rates, and place new demands on medical logistics. "The future battlefield is one of isolation, without the ability to evacuate casualties or get resupply," said the Commanding General of the U.S. Army Medical Research and Development Command (USAMRDC) in late 2021 [6].

Meeting the mission of CCC on the future battlefield will require new approaches to military medical research and new innovations to deliver advanced resuscitative care to the Warfighter at the POI. CCC equipment and technology must become smaller, lighter, more resistant to wear and prolonged periods of storage, and more effective. Central to this effort is the application of nanotechnology to CCC practices and products. Nanotechnology is the manipulation of matter at the atomic scale, with at least one dimension less than 100 nm

(or less than one billionth of a meter). While the concept of engineering at the nanoscale has been in existence since at least the 1950s, advances in computing power, manufacturing processes, and optics fabrication since the 1990s have driven great strides in scientists' ability to effect changes at an unfathomably small scale—the width of a human hair is roughly 90,000 nm [7].

The federal government and DoD alike have invested heavily in nanotechnology RDT&E over the past two decades, and current high-level policy documents continue to highlight the criticality of nanoscience, nanoengineering, and nanomanufacturing to U.S. national security [8]. Looking to accelerate the pace of innovation, the U.S. National Nanotechnology Initiative (NNI) was stood up in 2000 to guide a whole-of-government coordinating approach for the field. Given the great promise attendant to the nanoscale, creation of the NNI also generated, in some, an unrealistic view at the time that the field would deliver breakthroughs immediately. However—and especially in the medical field—those breakthroughs are increasingly coming to fruition. For example, recent work (2021) funded by the National Institutes of Health on skeletal muscle injuries—like a strained trigger finger—demonstrated the usefulness of a nanoengineered "bio-ink" to promote muscle recovery and reduce fibrosis [9]. Elsewhere, DoD and researchers at Rice University are working to manufacture nanomaterial-enhanced "smart helmets" and exoskeletons to better protect the U.S. Warfighter [10].

1.2 METHODOLOGY AND APPROACH

To produce this report, the Homeland Defense and Security Information Analysis Center (HDIAC) reviewed a broad array of scientific and engineering literature, including peer-reviewed publications, government reports, project reports from DoD-funded efforts, commercial pamphlets, and other sources of relevant "gray" literature. HDIAC also interviewed a dozen subject matter experts (SMEs) in medicine, engineering, nanoscience, and other relevant fields, as well as project managers and other DoD leaders who oversee research funding in nanotechnology or CCC. When possible, SMEs provided peer review of drafts of this report. Attendant to its goal of identifying the current state of the art, both the literature review and SME discussions focused on efforts from approximately 2019 to the present. Our findings assess what nanoenabled tools the DoD are currently using for CCC and what developments might be reasonably fielded in the near term. Therefore, we focused on products that are commercially available, currently being used in the field, or late-stage commercialization (nearing clinical trials, in clinical trials, or high technology readiness level [TRL]).

Most emerging trends convey research that is beyond basic and fundamental stages. Therefore, in the interest of time and focus, the following three topic areas will not be included in this report; however, they are extremely important, with the second and third topics holding enough volume to warrant their own individual reports:

1. Equipment used in field medical care (as it relates to engineered nanomaterials).
2. Canine combat casualty care.
3. Chemical, biological, radiological, and nuclear (CBRN) defense and mitigation.

CCC is a broad field that encompasses many other areas of medical practice and research, each of them extremely well developed and robust. In structuring this report, we reviewed the missions and focus areas of agencies that currently support CCC research and tactical medicine within the DoD enterprise. Based on that analysis, we concluded that the most logical set of categories must include the following:

- Hemorrhage Control and Fluid Resuscitation.
- Wound Management and Infection.
- Tissue Regeneration and Engineering.

- Neurotrauma and Pain Control.
- Advanced Medical Monitoring and Diagnostics.

To determine the best way to present this information from a research and development (R&D) perspective, we followed the flow of trauma management and grouped topics together that overlap (Figure 1-1).

1.3 STRUCTURE AND FINDINGS

The core content begins with Section 2, "Hemorrhage Control/Fluid Resuscitation," as this is the most important aspect of CCC and prehospital trauma care in general. Additionally, as it relates to nanotechnology research, many of the techniques found in this discipline can be seen as a foundation for nanotech research in other areas of CCC. Multiple products exist now to provide synthetic blood substitutes for CCC. Although none of them have yet entered clinical trials, they reflect a shift from just mimicking the function of blood to mimicking its structure + function; overall, all roads of relevant R&D in this area lead to the primary goal of creating a viable whole blood alternative.

Because the need to control bleeding and promote hemostasis goes hand in hand with controlling blood/fluid volume and bleeding, Section 3, "Wound Management and Infection," follows. This section also addresses infection in open wounds, such as those treated during conflicts in Iraq and Afghanistan, as such infections can come from a variety of sources (notably ESKAPE pathogens) and lead to life-long complications, such as osteomyelitis. It concludes with an overview of burn management, as burns are a unique type of injury that presents three main challenges—fluid loss, infection, and pain control—and offers a glimpse into tissue engineering, itself a microcosm of the challenges faced in CCC. Nanotechnology and the convergence of biotechnology are enabling a shift from purely passive dressings that merely cover wounds to more active and responsive dressings that actively promote wound healing, tissue growth, real-time monitoring of wound status, and antimicrobial therapy.

While more advanced care than what is provided on the battlefield, tissue regeneration (Section 4, "Tissue Regeneration and Engineering") has become an important part of CCC research because it can be viewed as an extension of wound care (i.e., a continuation of the healing process). Many of the necessary interventions needed to ensure successful longer-term outcomes should be performed as close to the time of injury as possible, hence, why it makes sense to discuss tissue engineering in this report. While many types of tissues are under evaluation for regeneration efforts, the most successful research to date has been seen with reengineering bone tissue. This is partly because bone is a naturally occurring nanocomposite, leading to an inherently smooth overlap from real to synthetic.

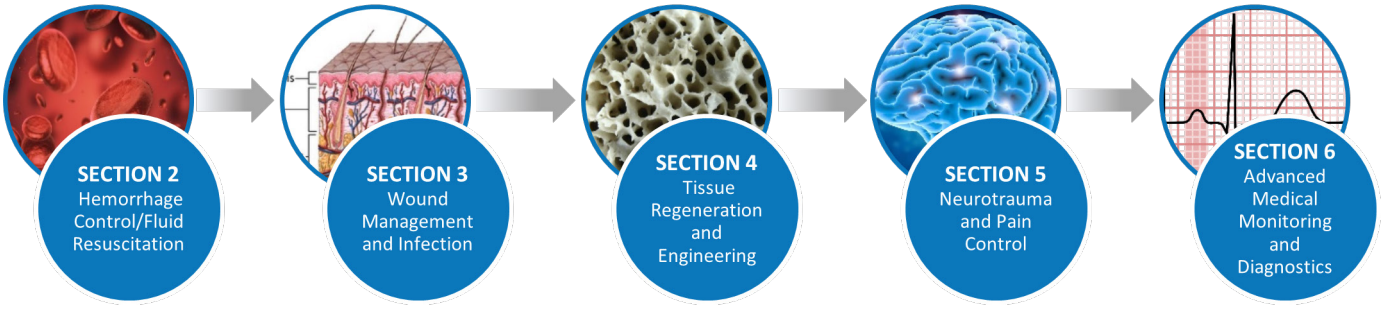


Figure 1-1. Five Core Content Areas and Content Flow *(Source: Gregory Nichols).*

Section 5, "Neurotrauma and Pain Control," focuses on brain and nervous system injuries, an extremely important part of modern CCC. It explores aspects that overlap with concepts discussed in Section 4 regarding tissue regeneration and Section 6, "Advanced Medical Monitoring and Diagnostics," especially from the perspective of using nanosenors to detect and mitigate brain injury. Nanopharmaceutical research for addressing pain control and traumatic brain injury (TBI) using intranasal delivery to cross the blood-brain-barrier and emerging methods to mitigate TBI and nervous tissue damage at the time of injury are also analyzed.

The core content of this report ends with Section 6, which links each of these sections together and mirrors the continuity of care found in modern CCC. Uses of nanotechnology to enhance medical monitoring during prolonged field care and en route care are discussed, as well as emerging areas of R&D for real-time biosensing, theragnostics, and converging technologies (e.g., internet of things and three-dimensional [3-D] printing). Among current and emerging trends, the use of graphene (GR) in nanosensors, implantable sensors for monitoring biomarkers in real-time, and a shift toward biosensing with smart textiles and self-powering nanogenerators are evaluated.

1.4 TRENDS AND CHALLENGES

Although CCC and nanotechnology are each massively broad fields with well-developed subdisciplines and bodies of knowledge, our research uncovered som e clear, current, and emerging trends within core areas (Table 1-1).

With new breakthroughs rapidly coming into view, the state of the art in nanotechnology's use in CCC is assessed next. Although discrete elements of CCC and other phases of military medical care receive significant research efforts, such attention is stovepiped from assessing more foundational work in broad fields like nanotechnology. Moreover, the

Table 1-1. Current and Emerging Trends for the Five Core Areas of Nanotechnology Research in CCC

Category	Current Possibilities	Future Possibilities
Hemorrhage Control/Fluid Resuscitation	• Platelets • Oxygen carriers (red blood cells [RBCs]) • Other hemostatic agents	• Whole blood alternatives
Wound Management and Infection	• Passive bandages and dressings • Bionanomaterials (chitosan) • Antimicrobial coatings/loaded nanoparticles (NPs)	• Real-time wound monitoring • Active/responsive dressings
Tissue Regeneration and Engineering	• Bone rebuilding	• Onsite tissue regeneration for all tissues
Neurotrauma and Pain Control	• Measure blast pressure • Rapid TBI biomarker assessment	• Intranasal delivery route (pain control) • On-site central nervous system injury mitigation
Advanced Medical Monitoring and Diagnostics	• Graphene-based sensors • Implantable/injectable sensors (glucose) • Sweat/interstitial fluid-based approaches	• Moving point of care (POC) to individual • Extending the capabilities of field medical personnel (theragnostics) • Real-time monitoring at the cellular level (prediagnostics)

changing nature of nanoscale R&D supports an updated look into its promise for delivering advanced CCC. In some taxonomies, what was previously viewed as a distinct nanospecific field is described variously as a subset of advanced manufacturing, advanced materials engineering, or bioengineering. Indeed, one of the highest overall trends identified in this report is the broad shift toward converging nanomaterials, biomaterials, and advanced (and/or additive) manufacturing techniques. In many fields, but with respect to CCC, much nanoscale R&D has shifted away from seeking an a priori nanocentric solution to a problem toward producing solutions that also utilize nanotechnology.

Additionally, external forces and breakthroughs in other areas of medicine may also be leaving their mark on the future of nanotechnology in CCC. For example, the COVID-19 pandemic has ignited a flurry of R&D in rapid clinical diagnostics development. Nanotechnology is being used to develop more accurate sensors to detect SARS-CoV-2 and other pathogens from a variety of sources. The use of lipid nanoparticles (NPs) in nanomedicine is clearly an emerging trend in the literature, and this will continue to be spurred on by the successful use of lipid NPs in COVID-19 messenger ribonucleic acid (mRNA)-based vaccines. Developments in diabetes care, from chronic wound management to continuous glucose monitoring, as well as advances in nanotherapeutic delivery systems in oncology could be adapted for use on the battlefield. Overall, nanotechnology development is making room for an easier crossover between the following core content areas of this report and CCC in general:

- Hemorrhage Control + Wound Management.
- Wound Management + Tissue Regeneration.
- Would Management + Advanced Medical Monitoring.
- Neurotrauma + Advanced Medical Monitoring.
- Neurotrauma + Tissue Regeneration.

In many ways, nanotechnology is helping to unite the fundamentals of CCC. However, to remain a successful technology and continue to adapt and change to the growing needs of medicine, these are the following core issues to overcome that plague most technological development:

- Cost compared to existing technology.
- Materials limitations due to current state of the science.
- Scalable production capacity due to lack of manufacturing need and ability.
- Innovation and commercialization of desirable products.

We identified several ways in which nano-technology is currently being used in aspects of CCC. However, most of these applications are still in their infancy, and only a handful have reached successful commercialization stages. As the field of nanotechnology continues to enjoy steady growth and converge with other technologies, the bound of the technology theoretically knows no limits but may run the risk of not having a clear, developmental pathway. With that in mind, there are several challenges we have identified that pertain to the specific use of nanotechnology by the DoD in CCC:

1. How do we translate nanoscience into treating combat wounds of the future?
    a. Burns – anticipated to be a major injury type in future conflicts.
    b. New injuries from new weapons – directed energy and kinetic.
    c. Old injuries from old weapons – e.g., artillery.
    d. Civilian casualties/humanitarian assistance – i.e., mass conflict abroad, Defense Support of Civil Authorities operations, foreign humanitarian assistance.
    e. CBRN threats in conjunction with traditional combat injury concerns – injuries and incapacitation from traditional and emerging weapons of mass destruction.

2. How do we not overengineer solutions? How do we ensure that nanotechnology solutions are not used just because they can be?
3. How do we do a better job of using nanotechnology appropriately?
    a. What safety and ethical issues still need to be addressed?
    b. Regulatory pathways.
    c. Long-term biokinetic modelling (especially with nanobiomaterials).
4. Privacy/data security regarding advanced detection, diagnostic, sensing, and monitoring technologies.
5. What lessons learned from nanotech applications in the following fields can be brought to CCC and how?
    a. Diabetes care.
    b. Oncology.
    c. Neurology/neurosurgery.
    d. Orthopedics.

1.5 SETTING THE STAGE

As the DoD shifts toward the wars of the future, its medical enterprise continues to keep pace with how best to address these new medical challenges. It is timely to reflect on the lessons learned of the past, while looking forward to how best new technologies can be used to enhance medical missions in an era of multidomain operations (MDO). Furthermore, to our knowledge, no sole source has summarized or comprehensively assessed the current and potential applications of nanotechnology in CCC. As a result, this report should be of particular interest to military medical personnel, academic and private researchers, and key decision makers in the RDT&E and DoD innovation enterprise.

SECTION 02 HEMORRHAGE CONTROL/FLUID RESUSCITATION

2.1 OVERVIEW

Over 90% of potentially survivable deaths on the battlefield (assessed during U.S. operations in Iraq/Afghanistan from 2003 onward) are caused by severe blood loss that could otherwise be treated with blood transfusions if blood products are readily available at the POI/POC [11]. Uncontrollable hemorrhage is the largest single cause of combat deaths and accounts for more than 80% of deaths on the battlefield [12]. Previous research has established that the use of hemostatic agents and fluid resuscitation play an extremely important role in preventing death from blood loss and trauma-induced coagulopathy [13]. The DoD has been interested in treating blood loss on the battlefield since World War II. However, delivering and storing blood and blood products in austere and remote environments have obvious and significant logistical challenges that are not easily overcome. Considering this, the DoD has invested heavily (since the 1970s) in developing semisynthetic and synthetic blood surrogate products as well as methods to provide reasonable blood replacements in combat environment.

Blood can best be described as a "fluid connective tissue where living cells are suspended in [a] non-cellular liquid matrix" [14]. Human blood has four key components—RBCs, white blood cells (WBCs), platelets, and plasma (see Figure 2-1). Together, these components are responsible for maintaining significant hemostatic functions in the body, including gas exchange (RBCs), immune surveillance (WBCs), clotting (platelets and plasma coagulation factors), and nutrition (plasma-borne components) [14]. The use of whole blood (all the components of blood found in a uniform ratio) in injury treatment has been associated with improved survival [11].

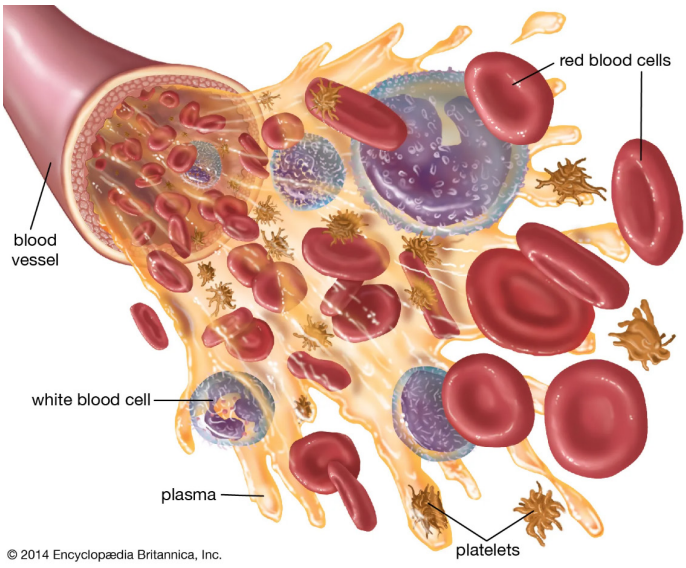


Figure 2-1. Major Components of Blood *(Source: Encyclopædia Britannica [15]).*

On the battlefield, the key goal of damage control resuscitation (DCR) is to restore homeostasis, leading to the prevention or mitigation of tissue hypoxia, the burden of shock, and coagulopathy [11]. DCR is most effective when lost blood is replaced with whole blood. When whole blood is not available, RBCs can be given to address

tissue hypoxia and oxygen debt. Platelets play an extremely important role in hemostasis, and their early use in treating hemorrhage has increased survival in trauma [11]. Plasma is the fluid in which the other blood components flow, but it also carries clotting factors and other necessary proteins for hemostasis. WBCs are not a component of interest for CCC. Advanced Trauma Life Support guidelines currently recommend that blood products, including plasma and platelets, be given early to bleeding patients while focusing on achieving hemorrhage control [16].

In terms of current blood logistics, the DoD is looking for the following characteristics in any blood surrogate product [17]:

- Freeze-dried with rapid reconstitution in deionized water.
- Small carry weight as lyophilized powder.
- Ease of portability without requiring cold storage and special conditions.
- Universally applicable without the need of type matching.
- Easy delivery of any product in the field.

Research regarding applications of nano-technology in designing blood replacements comprises two main areas—RBC surrogates and platelet surrogates. Additional hemostatic agents where very specific aspects of coagulation biology like improving fibrin stability, rendering von Willebrand Factor (vWF) response, etc., are being attempted with polymeric nanomaterials.

This section focuses on the most relevant and currently possible solutions for artificial blood products based on nanotechnology. A discussion of the most active areas of research in this field is presented (platelets, oxygen carriers, and whole blood), along with current examples nearing commercialization. Emerging techniques to develop these solutions and challenges in the field are also presented.

2.2 ARTIFICIAL BLOOD SUBSTITUTES

The R&D and commercialization paths of artificial surrogate products are very clear—all roads lead to whole blood. This goal could be realized in several ways. First, it can be a substitute that comes in a more traditional form that consists of multiple components (platelets, plasma, and RBCs) like natural human blood. Second, it could be a replacement that provides all the major functions of whole blood but exists as one integrated product. The first scenario is most likely, even with the challenges of developing a functional product with multiple components working in unison. The road to whole blood begins with developing blood components and other intrinsic factors that contribute to hemostasis and homeostasis. While this goal could be achieved without the specific use of nanotechnology or nanoscale components, most, if not all, of the most promising and successful developments to date are based on nanoenabled solutions [18].

2.2.1 Platelets

Platelets are anucleated blood cells primarily responsible for blood clotting and hemostasis. They stay in constant surveillance of the vessel wall and can rapidly provide a hemostatic response when needed. A loss of platelets due to trauma can lead to a variety of bleeding complications. Platelets are notoriously difficult to use in remote and austere environments because platelet suspensions have a shelf-life of only five days at room temperature [19].

Utilizing synthetic solutions rather than a natural platelet product is preferred for the following reasons [20]:

- Reduced likelihood of transmission of viruses and bacteria.
- Less likely to cause life-threatening complications like allergic or febrile reaction.
- Reduced alloimmunization after repeated platelet transfusion.

- Optimal storage properties.
- No need for blood group typing and reduced risk of side effects.

The interest in exploring nanotechnology for emulating platelet mechanisms in blood clotting and hemostasis is important because manipulating clotting on a nanoscale allows molecular scale biological interactions, which may not be possible with non-nanoscale blood technologies [17]. Early efforts in this area focused on replicating something that could plug an injury site the way that a platelet does. Current efforts seek to mimic multiple facets of coagulation mechanisms as much as possible. To be effective, synthetic platelets should have a combination of adhesion, aggregation, and procoagulant properties to mimic those properties in natural platelets [17, 20]. Parallel, significant interest in synthetic platelets substitutes not only explores hemostasis but also "large-scale preparation, minimum contamination risk via effective sterilization, shelf-life, no need for blood type matching, and reduced risks of biologic or pathologic side effects" [14, 20].

Platelets are microscale (2–3 μm in diameter), discoid, cellular entities that have a variety of surface glycoproteins and receptors present at different densities for specific biological functions. A variety of platelet-inspired systems has focused on mimicking platelets' biochemical mechanisms of injury site-specific adhesion, aggregation, and coagulation promotion via surface modification of microparticle and NP systems with platelet surface-relevant glycoproteins and ligands. These systems seek to simulate major hemostatic functions and activity of platelets on semisynthetic or synthetic nanotechnology platforms. The focus has not been necessarily to simulate the exact densities of interactions but rather the type of interactions [19, 21].

Researchers are currently pursuing the following categories of synthetic platelet substitute designs [14, 21–23]:

1. Fibrinogen-mimicking systems – micro- or NPs are surface coated with fibrinogen or fibrinogen-relevant peptides to amplify fibrinogen-mediated "aggregation" of active platelets via binding to platelet GPIIb-IIIa.
2. Mimicking adhesion mechanisms of platelets by modifying NP surfaces with recombinant GPIbα- or vWF-binding peptides (VBPs) to bind vWF and recombinant GPIa-IIa or collagen-binding peptides (CBPs) to bind collagen.
3. Combining platelet-inspired aggregation and adhesion mechanisms via heteromultivalent modification of NPs with VBPs, CBPs, and GPIIb-IIIa-binding peptides.
4. Decorating particles with fibrin-binding motifs to recapitulate the clot-retractive biomechanical properties of platelets.
5. Incorporating platelet-derived polyphosphate (PolyP) in NPs to modulate coagulation mechanisms.
6. Biointerfacing polymeric NPs with platelet-derived membranes to impart specific bioactivities.
7. Modifying NP surfaces with procoagulant lipids like phosphatidylserine (PS) for injury site-specific PS exposure to mimic the platelet's ability to amplify thrombin.
8. Focusing on incorporating platelet-inspired shape, size, and mechanical flexibility aspects to the platelet's design.

Representative products of these designs are depicted in Figure 2-2. Of all solutions, heteromultivalent modification of NPs with motifs mimicking platelet's adhesion and aggregation mechanisms seems to offer the most promise and is exemplified by the product SynthoPlate, a DoD-funded "platelet surrogate" design developed and patented by Sen Gupta's team at Case Western and now licensed to Haima Therapeutics for further translational development [24].

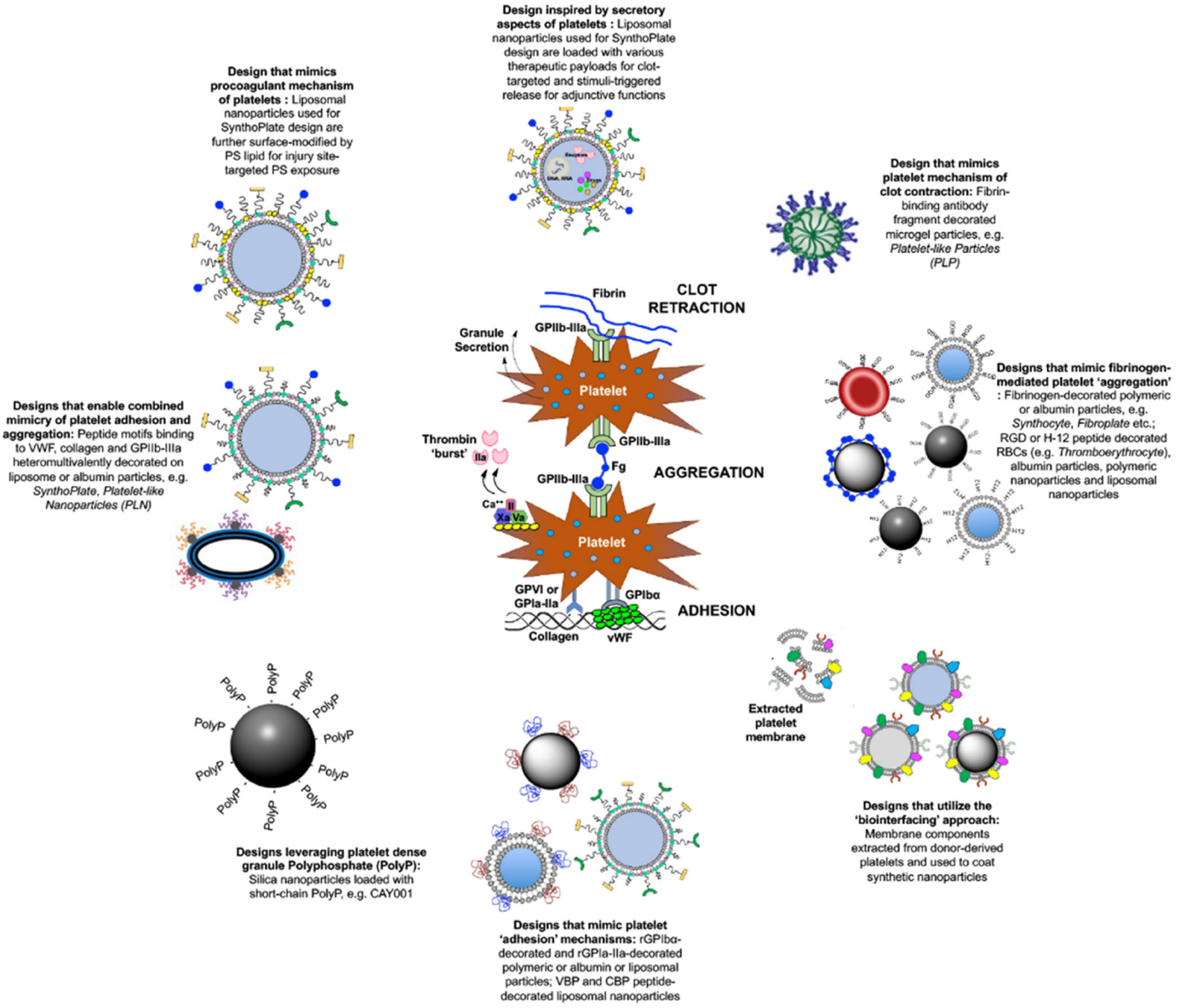


Figure 2-2. Platelet-Mimicking NP Designs *(Source: Haima Therapeutics [24], Provided With Permission From Dr. Anirban Sen Gupta).*

2.2.1.1 SynthoPlate

SynthoPlate, developed by Dr. Anirban Sen Gupta's laboratory at Case Western Reserve University and currently licensed to Haima Therapeutics in Cleveland, OH, is a novel artificial platelet system that involves decorating NP surfaces with a combination of vWF-binding peptides, collagen binding peptides, and active GPIIb/IIIa-binding fibrinogen-mimetic peptides (FMPs) to mimic the hemostatically relevant adhesion and aggregation mechanisms of platelets [17]. Haima Therapeutics is currently supported by multiple DoD awards (Peer Reviewed Medical Research Program, USAMRDC, and Combat Readiness-Medical Research Program mechanisms) and National Science Foundation (NSF) and National Institutes of Health (NIH) Small Business Innovation Research (SBIR) awards to continue developing SynthoPlate for the battlefield. SynthoPlate uses a heteromultivalent modification approach in its design. An overview of this technique is as follows:

"Heteromultivalent modification and integration of biochemical and biophysical parameters in many biological mechanisms, cell-cell interactions, and cell-matrix interactions are mediated by a concert of parallel heterotypic ligand-receptor interactions rather than a single type of ligand-receptor interaction. In case of blood cells, this is found extensively in the intercellular as well as cell-matrix interactions of platelets and WBCs. Recent advancement in nanomedicine research has focused on elucidating and exploiting these heteromultivalent interactions in enhancing the bioactive performance of nanoparticle systems. For example, WBC interactions with inflamed endothelium are mediated by a combination of integrin-based and selectin-based binding and rolling mechanisms. Platelet interactions at the injury site are mediated by a combination of vWF-binding, collagen-binding, integrin-binding, and selectin-binding mechanisms. Such combinations can be efficiently simulated on nanoparticle platforms by controlled co-decoration of the particle surface with multiple types of ligands. In carrying out such heteromultivalent decorations, researchers should ensure that the decorated motifs do not sterically interfere with each other, and this can be achieved by controlling the size as well as the spacer lengths of the conjugated motifs. Another emerging area of cell-inspired nanomedicine research is the utilization of cell-mimetic biophysical parameters, e.g., size, shape, mechanical modulus, surface charge, etc., combined with the cell-inspired biochemical (e.g., ligand-receptor interactions) parameters. Bottom-up fabrication techniques like self-assembly and emulsion-directed precipitation as well as top-down fabrication techniques like controlled lithography, hydrodynamic jetting, non-wetting templates, thermostretching, etc., can provide effective ways to incorporate such morphological and physico-mechanical parameters in design refinement of cell-inspired nanomedicine systems" [14].

SynthoPlate integrates the adhesion and aggregation functions of platelets without creating a thrombosis by using peptides instead of proteins, as the body tends to react less to peptides (Figure 2-3).

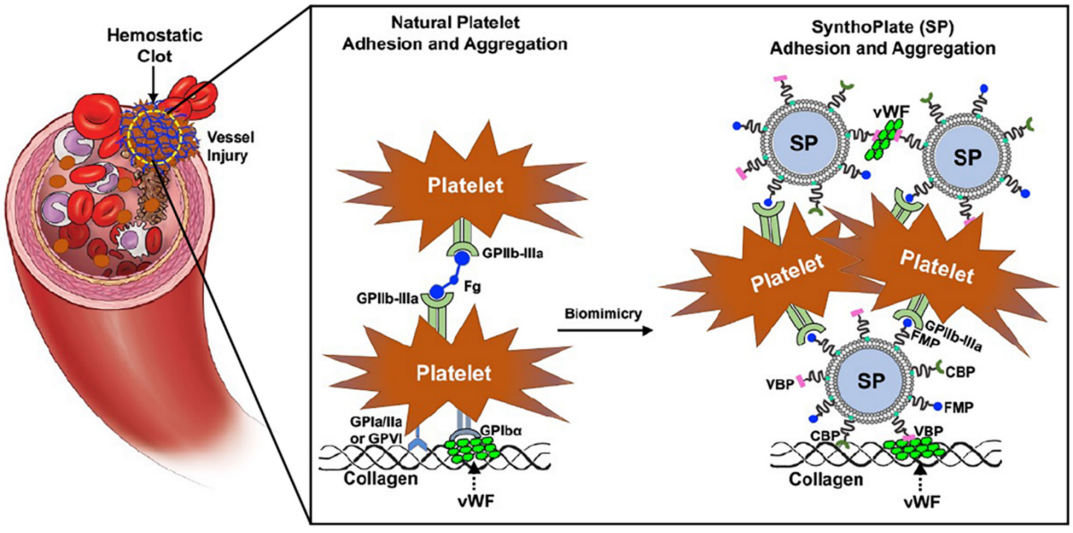


Figure 2-3. Schematic Representation of SynthoPlate Design and Mechanism *(Source: Image Provided With Permission From Dr. Anirban Sen Gupta).*

The product consists of two components: a biocompatible liposomal membrane with VBP and CBP to mimic platelet adhesion and a GPIIb/IIIa-binding FMP to mimic platelet aggregation [25]. SynthoPlate offers significant storage advantages to the Warfighter in remote environments, as it has a shelf life of six to nine months in aqueous suspension and can also be lyophilized for extended storage and on-demand aqueous reconstitution. It has been evaluated in small and large animal models of bleeding [26].

Ongoing and future development for SynthoPlate include creating a lyophilized version that could be rapidly reconstituted in saline for on-demand hemostatic resuscitation in remote and austere conditions, as well as integrating SynthoPlate with freeze-dried plasma (FDP) and lyophilized RBC surrogate like Erythromer to advance toward creating biosynthetic whole blood [17].

2.2.2 Oxygen Carriers

One of the primary functions of blood is to transport oxygen (O_2) from the lungs to the tissues of the body, where it can then be used in cellular respiration to make energy for powering key cellular activities. This function occurs in two ways. To a lesser degree, O_2 can be dissolved in plasma and transported throughout the circulatory system. However, the primary route of transportation for O_2 is via binding to hemoglobin (Hb), a heme-based protein within RBCs. Hb is a remarkable molecule because it has a higher affinity for O_2 in lung tissues, especially the alveoli, where the O_2 concentration is highest, and a lower affinity for O_2 in tissues of the body, where O_2 concentrations are the lowest. This mechanism makes it possible for Hb (via RBCs) to pick up O_2 in the lung capillaries, circulate through the body, deposit it in tissues, pick up waste materials—especially carbon dioxide (CO_2)—travel back to the lungs, and exchange the CO_2 for O_2. This process not only provides O_2 for cellular respiration necessary to make energy that powers most cellular functions but also helps to regulate body pH through the O_2-CO_2 exchange. Therefore, a traumatic injury that results in severe hemorrhage not only impacts circulatory blood volume (which can cause hypovolemic shock and lead to cardiac and renal impairment) but also severely impacts tissue oxygenation, metabolism, and homeostasis. Considering this, recreating the O_2 transport mechanism has been a key area of research in blood replacement therapy.

One of the leading methods of recreating this mechanism has been to isolate and manipulate Hb through either polymerization, PEGylation, or encapsulation. These molecules are known as hemoglobin-based oxygen carriers (HBOCs). HBOCs could be used to provide oxygenation when RBCs or whole blood are unavailable [27]. Research in this area goes back to the 1950s, when researchers began to develop HBOCs and tried to recreate RBCs. However, early designs failed, as noncell-based Hb tended to dissolve into component parts and absorb quickly into organs. To this day, no HBOCs are clinically approved for use in the United States, and only a handful have been approved for use elsewhere in the world. However, with the advent of nanotechnology-based techniques, some of the earlier challenges of working with Hb have been solved.

Developing HBOCs has been a main area of DoD research, especially for CCC programs. RBCs, the main carriers of oxygen in the blood, have a shelf life of 20 to 40 days, hence, the interest in creating a synthetic replacement. The latest generation of HBOCs is "focused on encapsulation of Hb within various micro- and nanocarrier vehicles, to more closely mimic the physiological encapsulated state of Hb in RBCs" [27]. As Dr. Anirban Sen Gupta explains [28]:

> *"Encapsulation has become an emphasized area compared to PEGylated or polymerized Hb [because] isolated 'cell-free' Hb tetramers rapidly disassemble to form dimers and monomers which can cause toxicity and thromboinflammatory issues. To resolve the above, cell-free Hb was conjugated to polymers (e.g., PEGylated Hb) or polymerized (e.g., HemoPure, BioPure,*

PolyHb, etc., designs). Even with the above polymerization approaches, there is heterogeneity in product molecular weight, such that low molecular weight fractions in the product may undergo clearance into tissues or kidneys and cause toxicity. A feasible way to avoid this is to encapsulate Hb within nanoparticles and microparticles, because these particles may not undergo rapid clearance, stay in circulation, and potentially enable oxygen transport via the encapsulated Hb."

Encapsulation is one of the leading methods for developing HBOCs, and a variety of current strategies exists (Figures 2-4 and 2-5).

2.2.2.1 ErythroMer

One of the most promising advancements in oxygen carrier research and development is ErythroMer (Figure 2-6), a bioinspired artificial RBC designed by KaloCyte (Baltimore, MD). KaloCyte has received both DoD and NIH funding to support the development and testing of ErythroMer.

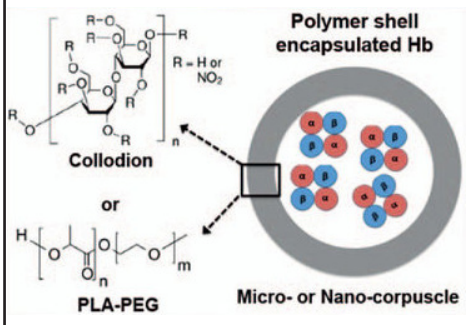
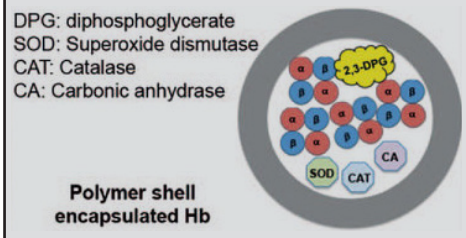
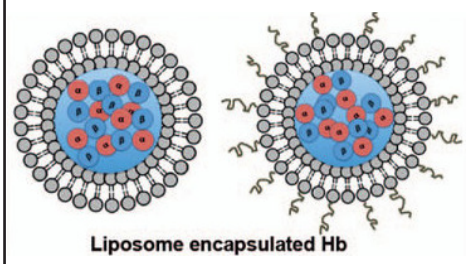
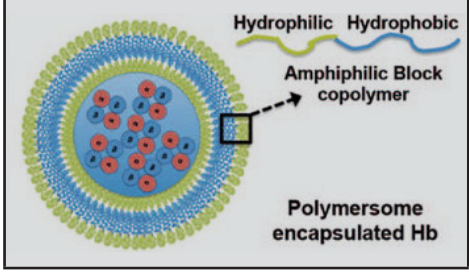
Encapsulated Hb-Based HBOCs	Materials Used	Representative Design Names
	Hb encapsulated within collodion (nitro-cellulose) membrane-bound or PEG-PLA polymer membrane-bound micro- or nanoparticles	Artificial Hb corpuscle
	Hb encapsulated along with various redox enzymes within collodion or PEG-PLA-based, membrane-bound micro- or nanoparticles	Artificial Hb corpuscle
	Hb encapsulated within submicron-size lipid vesicles (liposomes) and PEG-ylated liposomes (i.e., "stealth" liposomes)	Liposome-encapsulated Hb (LEH), "neohemocyte," "TRM-645 neo red cells," Hb vesicles (HbVs), etc.
	Hb encapsulated within submicron-size polymeric vesicles (ipolymersomes) made from amphiphilic block copolymers like PEG-PBD, PEG-PLA, PEG-PCL, etc.	Polymersome-encapsulated Hb (PEH)

Figure 2-4. Representative Approaches and Design Schematics for HBOCs *(Source: Sen Gupta [27])*.

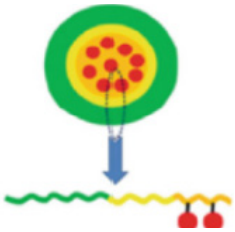
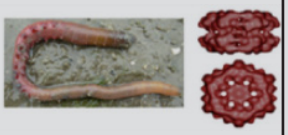
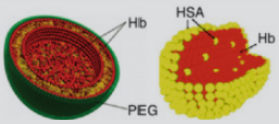
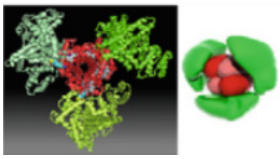
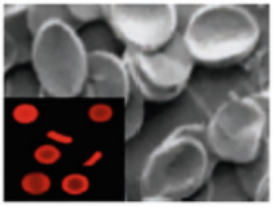
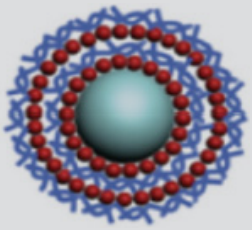
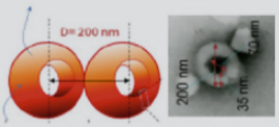
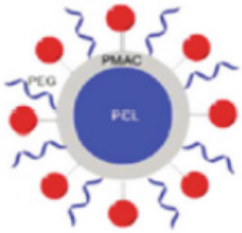
Novel Molecules and Designs for HBOCs	Product Names, Materials, and Design Approaches	Novel Molecules and Designs for HBOCs	Product Names, Materials, and Design Approaches
	HemoTech (HemoBioTech) Bovine Hb cross-linked intramolecularly with ATP, intermolecularly with adenosine, and further conjugated with GSH		Direct conjugation of Hb on the hydrophobic block of a block copolymer and subsequent micellization of the polymer molecules to form Hb-encapsulated micelle nanoparticles
	HEMOXYCarrier (Hemarina): giant extracellular Hb (3600 kDa) obtained from marine annelid		Hb co-precipitated with $CaCO_3$ or $MnCO_3$, stabilized by cross-linking (e.g., with glutaraldehyde) and further complexed with anionic proteins like HSA to form nano- or microscale clustered particles
	Core-shell structured protein clusters of bovine hemoglobin (Hb) and human serum albumin (HSA) by forming Hb-HSA via linkage of Hb surface lysines to HSA cysteine-34 using a-succinimidyl- ε -maleimide cross-linker		Mechanobiologic mimicry of RBCs where Hb is encapsulated within RBC morphology-mimetic, flexible, discoid, polymer-based microparticles formed by lithographic or template-induced printing techniques
	Template-induced, layer-by-layer (L-B-L) assembly of cationic Hb with anionic polymers like dialdehyde heparin (OHP), followed by dissolution of the template core		Erythromer (Nanocrit): hydrophobic tail conjugated amphiphillic polyethylene imine (PEI) molecules self-assemble with recombinant Hb, DPG, and antioxidants in reverse-micelle process to give nanobialys particle
	Conjugation of Hb on the surface of block, copolymer-based, core-shell nanoparticle structures		

Figure 2-5. Representative Schematics for Novel HBOC Molecules and Designs *(Source: Sen Gupta [27]).*

ErythroMer is a novel polymeric system using polyethylene imine modified with palmitic acid, forming toroidal-shaped nanoparticles (termed nanobialys, ~200-nm diameter). "These nanoparticles can encapsulate Hb and maintain a redox enzymatic environment for Hb activity by coencapsulation of 2,3-DPG and leuko methylene blue" [27].

According to KaloCyte [29], ErythroMer was designed with three primary goals in mind:

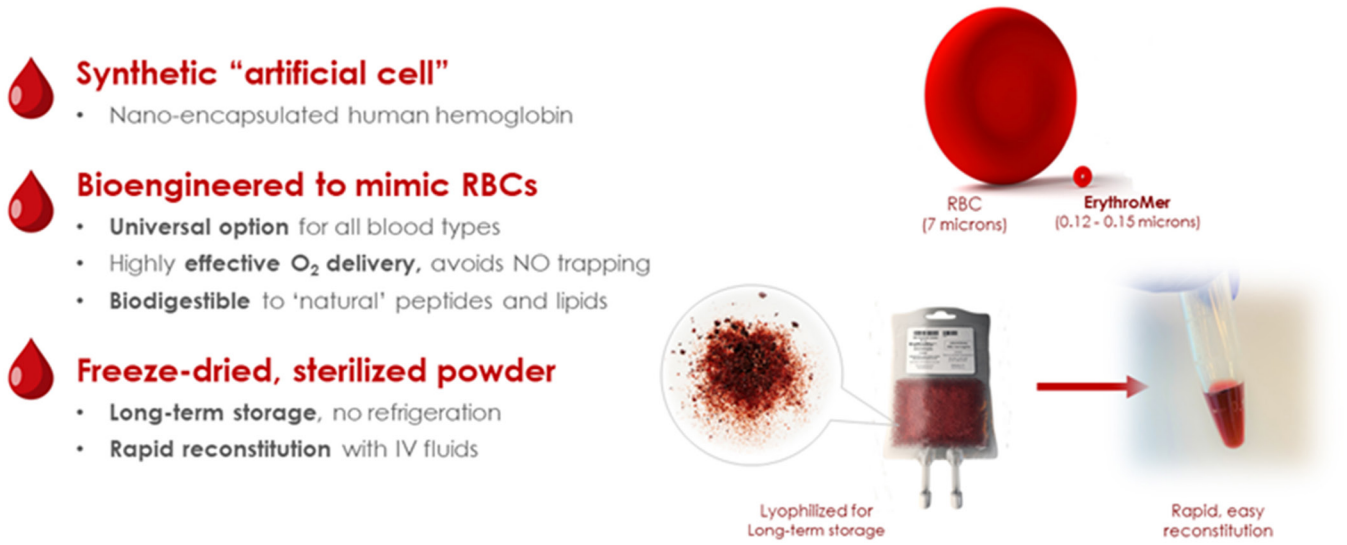


Figure 2-6. Product Features of ErythroMer *(Source: KaloCyte [29])*.

1. Normal RBC physiology emulation.
2. Robust storage capability and facile administration.
3. Cost-effective, efficient, and scalable formulation.

ErythroMer has a donut-shaped NP casing that mimics the outside of an RBC, which allows for "physiologically realistic gas exchange" [29]. The synthetic polymer shell eliminates the need to match blood type. ErythroMer can be lyophilized, increasing shelf life far beyond that of human blood. What sets ErythroMer apart from other solutions is that it exhibits "the first of its kind context dependent oxygen binding," similar to the actual oxygen transport mechanism of an RBC, which allows the distribution of oxygen in target tissues [29]. Closely imitating human physiology in this manner helps to reduce unintended vasoconstriction and hypertension, side effects indicated in previous blood replacement product designs.

2.2.2.2 $NanO_2$

Perfluorocarbons (PFCs) have been of keen interest for use in fluid resuscitation as well as in other areas of CCC and prehospital trauma care, e.g., TBI). One of the primary PFCs currently being explored for this purpose is dodecafluropentane emulsion (DDFPE) as a resuscitation fluid for treating hemorrhage shock and TBI [30]. The U.S. Army Medical Research and Materiel Command (USAMRMC) has deemed DDFPE "a better resuscitation fluid than volume expander" [31], which offers it key benefits for CCC use. Unlike other PFCs, DDFPE carries over 100 times more oxygen per unit weight, allowing it to be used in much lower doses than other oxygen therapeutics. The current leading product in this area is $NanO_2$, developed by NuvOx Pharma (Tucson, Arizona). $NanO_2$ is comprised of complex particles measuring ~250 nm in diameter and is "injected intravenously, flows through the lungs and picks up oxygen, then continues to flow through the blood and releases oxygen when in the presence of hypoxic tissue" [32].

2.2.3 Whole Blood

The U.S. Army Institute for Surgical Research (USAISR) Blood & Shock Resuscitation (CRT1) mission lists "engineered dried whole blood alternatives" as one of its key 2035 "paradigm-changing" efforts [33]. As just mentioned, all

roads in artificial blood product development are indeed leading to the development of a viable whole blood alternative. Stored whole blood lasts approximately one month at cold temperatures (21 days in citrate phosphate dextrose [CPD] bags and 35 days in CPDA-1 bags), hence, the great interest in developing a product that can survive in austere environments for long periods of time without cold storage and can be used when natural whole blood is unavailable. Whole blood substitutes must work in the body to be effective but must also be easy to mass produce. A traditional challenge with whole blood substitutes has been the use of HBOCs and platelet surrogates. While plasma can be freeze-dried and stored for long periods (one year or so), appropriate freeze-dryable RBC surrogate and platelet surrogate are the two factors that have not been fully achieved. (This is the current stage of the research funded by DoD.) Once this is done, these surrogates can potentially be integrated (FDP + RBC surrogate + platelet surrogate) into creating biosynthetic whole blood [28]. If newer products contain a Hb-based solution, there must be assurance that it can it be done in a safe way [18]. Currently, several potential solutions are under development.

The USAMRDC and Defense Health Agency currently support developing a freeze-dried whole blood product designed by DesiCorp (Louisville, KY). According to DesiCorp, it "can be infused into injured Warfighters immediately at the POI on the battlefield or to any other patients in remote or austere locations to treat traumatic blood loss" [34]. They use ultrasonic waves to load a protectant into RBCs to keep cells viable during the drying and rehydrating process and extend their shelf life. While not considered a true nanotechnology solution, the technology is using real RBCs, which range from 6 to 8 µm (6,000–8,000 nm).

Another developing solution is one proposed by several companies mentioned previously. Haima Therapeutics, KaloCyte, and Teleflex have future plans to explore integrating three of their products (SynthoPlate from Haima, ErythroMer from KaloCyte, and EZPlaz Freeze Dried Plasma from Teleflex) into a fully functioning whole blood mimic that could be converted to a lyophilized powder and reconstituted in the field when needed.

One of the leading programs dedicated to a viable development of a whole blood substitute is the Defense Advanced Research Projects Agency's (DARPA's) Fieldable Solutions for Hemorrhage with bio-Artificial Resuscitation Products (FSHARP) program, which "aims to leverage recent technical advances to meet the critical and immediate need for blood product alternatives" [35]. The program aims to leverage three key areas to address logistical challenges of using whole blood in forward settings:

1. New approaches to designing blood component substitutes.
2. Integrating those simulants into resuscitation products that approximate whole blood.
3. Manufacturing and stabilizing technology to ensure availability and functionality in challenging environments.

According to the FSHARP program manager, solutions are expected to "draw on recent innovations such as nanoparticle platforms for oxygen delivery, wound-sealing, or correction of disorders that accompany trauma" [36]. The ultimate goal is to develop bioartificial blood product substitutes that:

- perform the therapeutic functions of blood components important for resuscitation;
- integrate these products into formulations that can be coadministered to achieve near-parity to whole blood functionality, with no adverse effects;
- preserve processes that impart up to 6 months of shelf stability in a variety of expected operational conditions without a cold-chain requirement; and
- have manufacturing processes and technologies that enable quick, scalable, cost-effective, and consistent production.

2.3 CHALLENGES

There are several challenges to overcome before most of these solutions could ever make it to market [17]:

1. **Cost** – any solution cannot be cost prohibitive. For example, currently creating a platelet-inspired solution like SynthoPlate is cheaper than the cost to produce platelets.
2. **Regulatory Process** – There is no FDA precedent for guidelines as to what bar must be met. A product must work like nature. While any product should aspire to meet safety, purity, and potency of current good manufacturing practice, what exactly does this mean for artificial blood products and hemostatic agents?
3. **Immune Response** – Research has barely scratched the surface in this area. Everything should have the same response, but what happens with multiple doses over time?
4. **Biokinetic Modelling** – There are models but nothing for trauma, and this is the scenario where blood products would be used the most. There is currently not a good model available for traumatic coagulopathy.

Peng [37] sums up the current state of nanoenabled products in this area: "Some novel hemostatic agents based on nanomaterials are intriguing; however, it may be some time before they are brought to clinical trials."

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SECTION 03 WOUND MANAGEMENT AND INFECTION

3.1 OVERVIEW

Wound care is one of the most prolific areas where nanotechnology and engineered nanomaterials are being used for medicine, especially regarding their potential applications in CCC. Bandages and dressings are the centerpiece of development in this space, demonstrated by the fact that over 2,000 products exist. This is a highly competitive space, with the largest market share only being 10% (compared with 80% in other markets) [38]. This competition tends to spark innovation and easier adoption than other technology areas. One of the key drivers of R&D in nanotechnology for wound dressings (and to a larger degree, most wound care products) is innovation in nearly all major research universities, especially those with an affiliated medical school.

The flood of R&D in this area and the large number of commercially available products stem from a low barrier to entry due to the following [38]:

1. A huge market that can absorb consistent expansion.
2. A relatively easy regulatory process to navigate (Food and Drug Administration [FDA] 510(k) for medical devices).
3. A lack of need for large amounts of experimental and clinical data.
4. A class of products that only needs to demonstrate incremental improvement from previous iterations to enter the marketplace.

One final point that also explains the ever-expanding introduction of nanoenabled products in wound care is that many things developed for and used in wound care can, in some way, apply to other key areas of CCC—resuscitation, hemostasis, and tissue regeneration. These areas all have some overlap with commonly used materials and fabrication processes. Hence, nanotechnology in wound care can be viewed as a foundational space upon which other more advanced and complex applications may be derived.

The whole purpose of wound care is to promote wound healing. It is important to remember that the basic physiological process of healing occurs no matter what, but nanotechnology and nanomaterials can be used to enhance this process. Wound healing occurs in four main stages [39]:

1. **Hemostasis** – platelets aggregate and clots form to control bleeding.
2. **Inflammation** – biological mechanisms actively work to reduce microbial presence and prevent infection.
3. **Proliferation** – many different types of cells accumulate to the area and produce granular tissue.
4. **Remodeling/Maturation** – wound fully closes, fibroblasts work to form new skin, and scar forms.

Hamdan et al. [40] describe commonly used types of nanomaterials for each stage in the wound-healing process (Figure 3-1). The goal of using nanomaterials in wound management is to improve the efficiency,

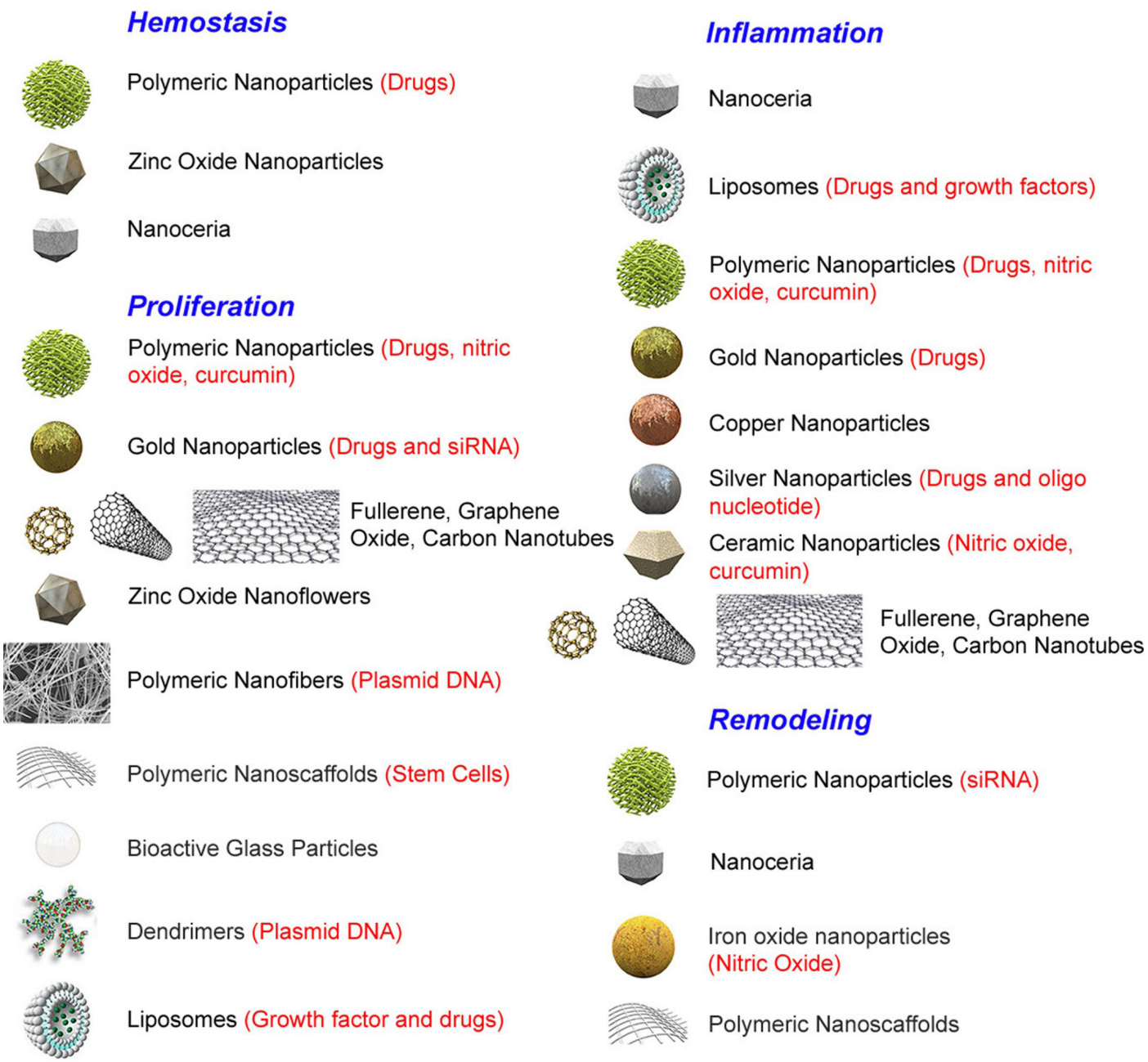

Figure 3-1. Schematic Representation of the Nanotechnology-Based Therapies Employed in Wound Healing *(Source: Hamdan et al. [40])*.

efficacy, healing time, and outcomes of one or more of these phases or the process overall.

At present, there are two core strategies for using nanomaterials in wound healing (Figure 3-2) [41]. First, some nanomaterials have inherent properties that are beneficial to promoting various aspects of wound healing (hemostatic properties, tissue regeneration properties, and anti-infective properties). Devices can be manufactured directly from these nanomaterials, or these materials can be embedded directly into other materials to create reactive surfaces. Second, nanoscale materials can be used to create nanocarriers that deliver therapeutic agents to target sites. Considering this approach, Figure 3-2 outlines the different types of possible therapeutics to be delivered by key nanomaterials under research for wound-healing management [42].

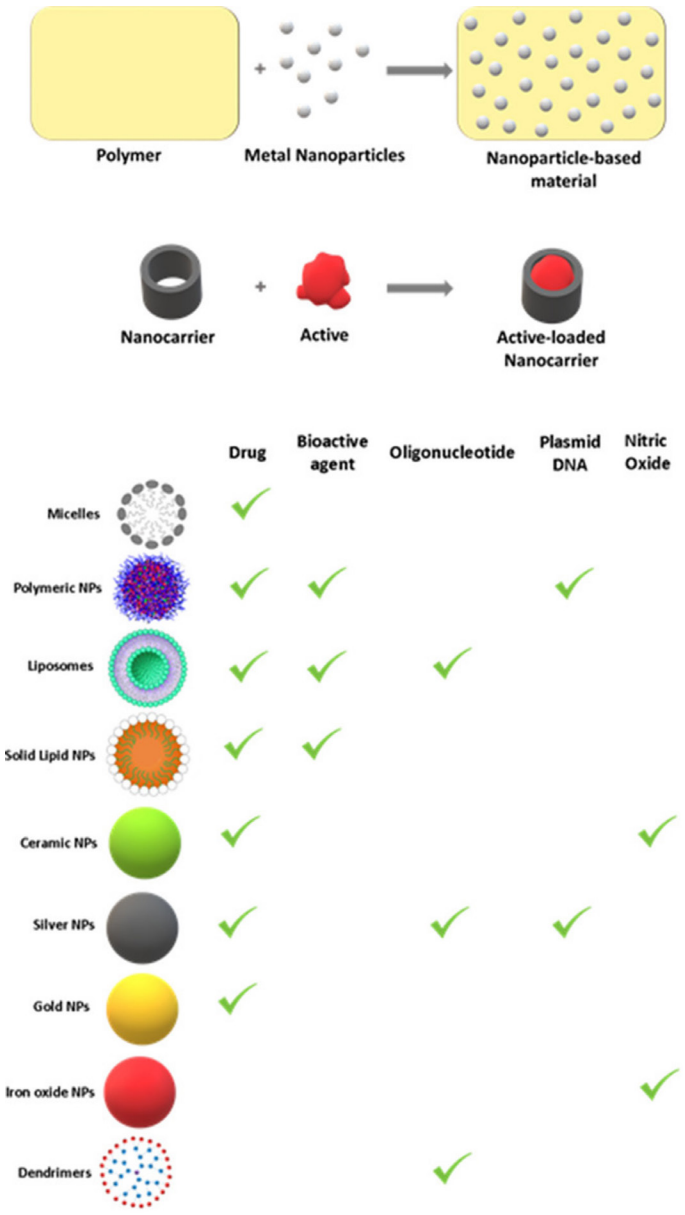

Figure 3-2. Two Main Strategies for Wound Healing (Upper: Preparing Organic Inorganic Hybrid Nanocomposite Embedding NPs in Polymers; Lower: Encapsulating Actives Into Nanocarriers) *(Source: Barroso et al. [42]).*

To make the vast world of nanotechnology in wound care more accessible, the discussion can be focused on appropriate applications relevant to combat, trauma, and prehospital situations. Additionally, wound management can be viewed in four relatively neat but interrelated areas—wound closure, bandages and dressings, infection control and prevention, and burn care.

3.2 WOUND CLOSURE

Once bleeding has been controlled, some wounds can be closed to protect them from further damage and infection. This is done for wounds where adjacent skin can be closed over the wound using tissue adhesives, sutures, and staples. Sutures have been used in closing wounds for thousands of years. Properties of effective suture materials include sterility, biocompatibility, flexibility, and intrinsic good mechanical strength [43]. Recently, there has been an interest in exploring nanometals as suture materials and a trend to add bioactive substances to polymers to achieve functional needs [44]. Two methods for adding nanomaterials into suture materials exist—electrospinning and coating/dipping. Xu et al. [44] provide an excellent and comprehensive overview of electrospinning for suture materials in Table 3-1, which summarizes bioactive materials that have been added to electrospun nanofibers. Triclosan and silver (Ag) NPs are the most used agents for antibacterial sutures *S. aureus and E. coli* [43], as demonstrated by their use in both electrospun and dipping applications. El Baher Dhafer and Debbabi [43] created an effective antibacterial suture through the development of a polypropylene suture coated with a blend of Ag NPs and carboxymethylpullulan, a derivative of the polysaccharide pullulan with excellent biocompatibility. Alirezaie et al. [45] demonstrated an effective use of coating silk sutures with Cefixime NPs, a broad-spectrum, third-generation antibiotic.

Another method for wound closure is the use of tissue adhesives. Yang [46] notes that tissue adhesives should have proper durability and stability for a given period. Many tissue adhesives have challenges like weakened adhesion and low biocompatibility, and several methods of using nanomaterials and/or biomaterials are being explored to overcome these challenges. Hydrogels could be ideal but tend to be fragile. Several methods have been developed to make hydrogels tougher for adhesive applications, including using quercetin-assisted, photo-radical chemistry;

Table 3-1. Bioactive Substances in Nanofiber Solutions *(Source: Xu et al. [44]).*

Bioactive Substances	Polymers	Characteristics	Preparation Method
Silver Nanoparticles	PGA-PLGA	Significant antibacterial effect, biocompatibility, and degradability	Blend electrospinning
Triclosan	Polylactose 910	Effective antibacterial avoidance of wound infection	Coating
NO	Acrylonitrile-co-1-vinylimidazole (AN/VIM)	Maintain good mechanical properties, antibacterial, and promote healing	Melt spinning
GO	PVA	Good antibacterial properties, low cytotoxicity	Blend electrospinning
Growth Factor (VEGF/bFGF/TGF-β)	RSF/BAMG, PCL/collagen, PLGA	Promote cell adhesion and value-add, promote the regeneration of new blood vessels	Coaxial electrospinning
Curcumin	PEG, PLA, and PCL	Good chemical stability, low toxicity, antibacterial, and healing	Blend electrospinning
Heparin	PLGA, PEO, and PgP	Reduces platelet adhesion, antithrombosis	Blend electrospinning
Aceclofenac/Insulin	PLLA/PLGA	Promote epidermal hyperplasia, cell adhesion migration	Blend electrospinning
Chitosan/Tetracycline Hydrochloride	Silk	Antibacterial, bleeding	Blend electrospinning

using Ag-lignin NPs to trigger dynamic redox chemistry; and creating self-healing hydrogels [46]. Additionally, biomimicry is often an effective strategy for identifying solutions from the natural world that may improve biomedical applications. Several studies have shown that polydopamine (PDA), a compound found to be critical for mussels to adhere to underwater surfaces, may be an effective additive in tissue adhesives [47]. Adhesives need to interact with the moist surface of a wound; using the natural mechanisms of how mussels do this could be achieved by coating nanomaterials with PDA to change or improve their properties. PDA is inert and nontoxic. Several studies have shown PDA-coated surfaces demonstrate improved adhesion upon cell contact compared to uncoated surfaces [47].

3.3 BANDAGES AND DRESSINGS

3.3.1 Overview

The use of nanomaterials to create bandages and dressings is one of the most prolific areas not just in wound care but all of healthcare. The whole point of developing a dressing is to repair a wound better and faster. Some of the key characteristics of an ideal dressing are as follows [48, 49]:

1. Prevent further damage.
2. Maintain humidity/ensure some moisture content.
3. Allow gas exchange.
4. Biocompatible and nontoxic.

5. Clear wound of debris and exudate.
6. Nonadhesive and nonshedding.
7. Allow for heat insulation.
8. Antimicrobial.
9. Hemostatic.
10. Comfortable/fit wound surface.

Current strategies of developing bandages and dressings using engineered nanomaterials are typically one of two approaches [48]. Nanomaterials can be used to enhance the bioactivities of current types of dressings, or they can be used to expand the range of applications of dressings (both existing and emerging). Dressings can be divided into three types [4]. Traditional dressings are typically some type of gauze usually made from cotton or some other natural fiber material. Biomaterial-based dressings are an emerging typemade from tissue derivatives or other non-traditional types of biomaterials. Finally, artificial dressings are those made from new materials and/or exist in a nontraditional form. These can include films, membranes, foams, gels, composites, and sprays—the area where much nanotechnology research for dressings currently exists (Table 3-2). While traditional dressings just cover and protect a wound, more advanced dressings actively interact with the wound and provide an enhanced environment for healing [48], representing a shift from passive to active dressing designs.

Table 3-2. Advantages, Disadvantages, and Suitable Conditions of Dressing Forms in Modern Medicine *(Source: Shen et al. [48]).*

Dressing Types	Advantages	Disadvantages	Suitable Conditions
Hydrogels	• Good absorption of exudate • Good moisturizing properties • Have a cleansing effect • No reoccurring mechanical damage • Self-adhesive • Concealed appearance • Good antibacterial properties • Accelerated wound healing	• Poor ability to absorb exudate • Higher costs • Possible allergic reaction	• Pressure ulcers • Surgical wounds • Burns • Radiation dermatitis • Diabetic foot ulcer
Nanofiber Mats	• Good antibacterial properties • Effective control of local wound infection • Good absorption of exudate • Accelerated wound healing	• Cytotoxic risk • Prone to allergic reactions • Higher production cost	• Burns and scald • Localized trauma infection
Films	• Good antibacterial properties • Good moisturizing properties • Self-adhesive	• Poor mechanical properties • Higher costs	• Epithelializing wounds and superficial wounds with limited exudate • Chronic venous ulcer • Radiation dermatitis
Membranes	• Good haemostatic effect • Promotes granulation tissue formation and self-decomposition of necrotic tissue • Good antibacterial property	• Poor ability to absorb ooze • Higher production cost	• Chronic venous ulcer • All kinds of dermatitis and eczema
Sponges	• Good absorption of exudate • Low permeability • Good antibacterial properties • Thermal insulation	• Excessive absorption • Higher costs • Inconvenient to observe	• Infected wounds • Diabetic foot ulcer • Medium to heavily exuding wounds • Venous ulcers

3.3.2 Polysaccharides and Chitosan

One of the leading developmental trends in nanomaterial-based dressing design is the use of polysaccharides to create or enhance dressings. Polysaccharides are widely used in wound dressings for several reasons [48]:

1. They are nontoxic.
2. They provide a framework for storing other materials and additives.
3. They have excellent biocompatibility with human tissues.
4. They support bioadhesion for healing and tissue regeneration.

An extremely common polysaccharide used in manufacturing dressings is chitosan (CS), which is a derivative of chitin. Chitin has poor solubility and is not easy to process; therefore, CS is the clear choice for its ease of processing. CS can be used as a structural component and as an active ingredient. It is biocompatible and biodegradable and possesses a wide host of desirable bioactive traits (Table 3-3). It is good for casting films, membranes, and electrospinning into nanofibers [48]. CS also offers many advantages for making smart hydrogels/injectable hydrogels and self-healing hydrogels. Although not nanoscale, several chitin- and chitosan-based dressings are commercially available, including Celox, Chitopak, Chitoflex, Tegasorb, and HemCon, which was funded by the U.S. Army and approved for use by the FDA since 2003.

Alginate is another commonly used polysaccharide for wound dressings. Several dressings currently exist on the commercial market, including Algivon, Sprasorb A, Algicell, Guardix-SG, SeaSorb, and Tromboguard. Alginate is a salt of alginic acid mainly found in the cell walls of brown algae [48]. It is particularly favored for use in wound management, as it is highly compatible with human tissue and has desirable properties for thickening, stabilizing, gel forming, film forming, and fiber spinning. Alginate dressings are used as barriers or drug carriers. Recently, several papers illustrated a trend toward research interest in creating alginate nanofibers, particularly blended with polyvinyl alcohol (PVA) or polyethylene oxide [48, 50, 51].

Table 3-3. Bioactivities of CS That Facilitate Wound Healing and Their Mechanisms *(Source: Shen et al. [48]).*

Bioactivities	Mechanisms and Hypotheses
Antibacterial	No definitive conclusion yet. The main hypotheses include: (1) adheres to and electrostatically disrupts bacterial cell walls and cell membranes, (2) chelates trace metal cations leading to potential imbalance, (3) interacts with intracellular targets to inhibit protein synthesis, and (4) deposits on bacteria and affects metabolism.
Anti-inflammatory	Induces increased levels of anti-inflammatory cytokines, such as IL-10 and TGF-β1, and decreased levels of pro-inflammatory cytokines.
Antioxidant	It is achieved by donating hydrogen atoms. The amino and carboxyl groups of CS stabilize free radicals.
Promotes Tissue Regeneration	Modulates growth factors to promote macrophage transfer to wounds, fibroblast proliferation, proteoglycan and collagen synthesis, and angiogenesis.
Haemostasis	Promotes the aggregation of platelets and red blood cells and their adhesion to tissues to form clots.
Scar-Free	Dependent on its cationic properties. CS inhibits the production of type I collagen in wounds, promotes the production of granulation and epithelial tissue, as well as reducing wound contraction, thereby reducing scarring.

As previously mentioned, electrospinning nanofibers from CS is a popular use of the material in wound dressings. According to Shen et al. [48], "nanofibers are wire-like materials with a certain aspect ratio at the nanometer scale." Electrospun fibers offer good mechanical properties and tissue regeneration capabilities because they are porous and hydrophilic, which promotes adhesion of fibroblasts. However, polysaccharide-based nanofibers can dissolve rapidly and are often stabilized with other synthetic or natural polymers. Polysaccharide nanofiber membranes, such as those made from CS, also promote water vapor transport and exudate absorption capacity. These characteristics in wound healing occur because CS (and other natural polysaccharides) nanofiber mats and scaffolds mimic the environment of the extracellular matrix (ECM). Shen et al. [48] also observed that the porous nanostructure of nanofiber mats allows uniform and vigorous drug loading, high encapsulation rates, and prolonged sustained release. Table 3-4 presents an overview of polysaccharide-based nanofiber dressings.

Although CS is an important material for design in wound care management, there are some barriers to longer term and more widespread use: (1) lack of prospective clinical trials and (2) unknown

Table 3-4. Summary of Raw Materials and Characteristics of Marine Polysaccharide-Based Nanofiber Dressings *(Source: Shen et al. [48]).*

MPs Component	Other Main Components	Active Agents	Biological Activities	Other Features
CS	Polyvinylidene fluoride Polyhydroxybutyric acid	Gentamicin	Not tested	Double layer drug delivery Efficient drug delivery Strong mechanical properties
CS	PVA Starch	—	Antibacterial Promotes tissue regeneration	High water vapour transmission rate to provide a moist, well-oxygenated, wound-healing environment Low cytotoxicity
QCS	Collagen PCL PVA	—	Haemostatic, antibacterial Anti-inflammatory Promotes tissue regeneration	—
CS	PCL	Human granulocyte, colony-stimulating, factor-loaded CS NPs	Anti-inflammatory Promotes tissue regeneration	The stent promotes stem cell adhesion and proliferation, sustained slow release
CS	PCL PVA Polycaprolactone	Melatonin	Anti-inflammatory Promotes tissue regeneration	Three layers of nanofibres Hydrophilic effect
CS	PVA Carbopol Polycaprolactone	Curcumin Mesenchymal stem cells	—	Promotes tissue regeneration
Alginate	WPU CaCl	—	Not tested	Effective drug delivery High mechanical strength
Alginate CS	Gentamicin	—	Antibacterial	Effective drug delivery Promotes tissue regeneration
Alginate	PUL	PL	Anti-inflammatory	High mechanical strength
Alginate	TOBC	Zn2+	Antibacterial	High mechanical strength
Alginate	PVA	Spider silks	Anti-inflammatory	Effective drug delivery Promotes tissue regeneration
Alginate CS	PCL Lumi	Doxycycline, PEO	Not tested	Strong, wet tissue adhesion High mechanical strength Effective drug delivery
Alginate CS	Glutaraldehyde polylysine	—	Promotes tissue regeneration	High water vapour transmission rate to provide a moist environment Effective drug delivery

metabolic pathway in vivo (potential risk of cumulative toxicity). Just as CS has become a preferred alternative to chitin, a new derivative of CS, carboxymethyl CS (CMC) is being explored for wound-healing applications, especially as an injectable hydrogel [48]. However, CMC is still in the laboratory stages, as it is difficult to process but does offer little-to-no toxic effects.

3.3.3 Hemostatic Dressings

Hemostatic agents are additive or nonblood products used to achieve clotting and control life-threatening, typically noncompressible hemorrhage. Blood products can be one part of that equation, but another class of agent also exists—those used to primarily induce hemostasis and achieve temporary clotting and hemorrhage control. According to Peng [37], the following are characteristics of an ideal hemostatic agent for battlefield hemorrhage control:

- Quick and effective control of bleeding in a wide range of conditions and from a variety of wounds within 2 minutes, even when applied to an actively bleeding site through a pool of blood.
- Sustainable hemostasis duration for several hours infused on the battlefield, reflecting delayed evacuation.
- Easy removal without leaving residues or no need for removal because of biodegradation; ready to use with little training and preparation.
- Easy administration, even by a layperson under austere conditions.
- Ease of manufacture and sterilization and low cost.
- Simple storage and high portability; prolonged stability (>two-year shelf life), even under extreme conditions (–10 to 55 °C).
- Good biocompatibility with no adverse effects on healing and no thromboembolic complications.

No hemostatic agents currently meet all these requirements, but the selection of a suitable material should be based on the probability of success in vivo, stability, ease of use, and ease of manufacturing [37].

Many hemostatic agents are used in conjunction with bandages and dressings, so there will be some overlap with hemostatic dressings discussed here and dressings that promote wound healing and tissue regeneration in sections that follow. One of the leading products in the area, and one that has been in commercial use for some time, is QuickClot Combat Gauze (Teleflex – Wayne, PA). The third and fourth generations contain kaolin, which is an "aluminosilicate nano-particulate which has been shown to accelerate the body's natural coagulation cascade" [52].

Most nanobased hemostatic agents are still experimental and unavailable for commercial use and will likely not be approved for human use soon. Those being developed tend to come in three forms—self-assembling peptides, cryogels, and solutions based on nanobridging. Behnke and colleagues reported on a self-assembling peptide that created a nanofiber barrier that achieved "hemostasis for internal bleeds…in less than 15 seconds in all experimental models" [53]. Peng [37] describes research regarding a "16-amino-acid self-assembling peptide" called RADA16-I, which can be incorporated into standard bandages and wound dressings via a layer-by-layer assembly. The peptide nanofibers generate nanofiber-based clots when exposed to physiological conditions, and porcine models demonstrate a promising approach using the peptide in conjunction with commercially available gauzes, even in harsh temperatures (~140–180 °F). Carbon nanotubes (CNTs) have been used to construct cryogels with "robust mechanical strength, fast shape recovery, and instantaneous and high blood absorption capacity" and "show better blood-clotting ability and higher blood cell and platelet adhesion and activation than available sponges and gauzes" [37]. Murine models have demonstrated better hemostatic effect with this CNT-based cryogel than

with a common product, such as Tegaderm. This cryogel performed better than gelatin sponges in noncompressible hemorrhage models and offered "even better hemostatic effect than Combat Gauze in a standardized liver bleeding model" [37]. Nanobridging is the process of spreading a droplet of a NP solution over the wound surface of damaged tissue. Meddahi-Pelle developed a solution including silica and iron oxide NPs that provides wound closure after 1 minute when manual pressure is applied [53].

3.4 INFECTION CONTROL AND PREVENTION

Infection control is an extremely important aspect of wound healing since open wounds are exposed to the environment and are more susceptible to colonization by pathogens and infections that may inhibit tissue remodeling, which prolongs and complicates wound healing. From a DoD perspective, over 30% of all combat wounds become infected, and that percentage increases the longer a wounded service member remains in transit [54]. Prolonged exposure to pathogens and source material following polytraumatic injuries can lead to long-term complications, such as osteomyelitis.

Since the discovery of penicillin, antibiotics have been the gold standard to treat most infections, including those incurred on the battlefield. However, the overprescribing and inappropriate use of antibiotics has also led to an increase in multidrug-resistant strains of bacteria, rendering many frontline antibiotics useless. As medical professionals scramble to address these threats, nanotechnology has offered a potential solution for treating and preventing infection in wounds.

Nanobased therapeutics offer several advantages to traditional antimicrobial therapies [55, 56]:

- Fewer side effects than conventional antibiotics.
- Enhanced interactions between bacteria or change pathway of the drug to improve antibacterial effects.
- Increased drug concentration at infection sites.
- Improved drug penetration into tissue barriers and bacterial biofilms to overcome resistance.
- Improved stability and prolong half-life of drugs.

These effects can be achieved by several different types of nanomaterials. The best approach to discussing the use of nanomaterials in antimicrobial therapy for wound infections is to focus on types of nanomaterials used and their benefits, beginning with polymer NP solutions.

Polymer micelles are one of the most studied types of nanomaterials for delivering antimicrobial agents, especially hydrophobic antibiotics, antimicrobial peptides (AMPs), and Ag NPs. Bioactive enzymes can also be loaded into polymer vesicles to combat bacteria [57]. Dendrimers can be loaded with antibiotics, AMPs, Ag NPs, and metal oxide NPs to create antimicrobial agents. Nanogels are structures composed of cross-linked hydrophilic or amphiphilic polymer networks. These are specialized types of hydrogels that incorporate NPs in them. Hydrogels have been used in antimicrobial applications because of their 3-D structure and high-water content. Nanogels, in general, have been targeted for drug delivery because they are flexible, stable, and have a high loading capacity. Antimicrobials such as berberine, cyclodextrin, tetracycline, lincomycin, and Ag NPs have been loaded into nanogels [57]. In addition, ciproflaxin, silver sulfadiazine, tetracycline, and gentamycin have been administered using polymer delivery devices [58].

One of the key classes of NPs being explored for antimicrobial therapy includes metals and metal oxides. Leading candidates are Ag, gold (Au), copper (Cu), zinc oxide (ZnO), and titanium dioxide (TiO_2), with Ag NPs being far ahead of the group. Metal NPs have characteristics like dimension and architecture, surface functionalization, zeta potential, and polydispersity index that make them excellent candidates as antimicrobial agents in wound dressings [58, 59]. Different metals target different functional groups' cellular

components differently to cause a variety of destructive mechanisms at target sites, including, but not limited to, membrane damage, altered membrane potential and solute transport, protein dysfunction, electron transport chain disruption, deoxyribonucleic acid (DNA) damage, inhibition of DNA replication, and redox imbalance [60]. The ability of metal NPs to disrupt microbial activity is caused by simultaneous release of metal ions and the intrinsic property of the NPs.

Silver is an extremely effective treatment against microbial growth. Ag NPs are potent, even at low concentrations, due to their increased surface-to-volume ratio [59, 60]. They have been explored extensively for wound infection. Their ubiquitous use in wound dressing is well documented. Several wound care products continuing to use nanosilver are currently available in commercial markets. Representative examples include ACTICOAT (Smith + Nephew – London, UK), Suprasorb A + Ag Lohmann & Rauscher International – Neuwied, Germany), and Promogran Prisma Matrix (3M – St. Paul, MN). Dressings with Ag NPs have been shown to accelerate wound healing by downregulating metalloproteinase and demonstrate an anti-inflammatory response through controlling the expression of TNF-α [60]. The primary antimicrobial mechanisms of action for Ag NPs are the creation of sulfuric bounds with bacterial cell membranes or thiol groups of enzymes, leading to apoptosis [58]. Ag NPs also interfere with DNA synthesis during cell division. They combine easily with other types of materials, including biomaterials like CS, alginate, and other pharmaceuticals. For example, many of the dressings mentioned previously contain a combination of Ag NPs with a biomaterial foundation. Studies have demonstrated that Ag NPs combined with tetracycline can significantly reduce bacterial contamination and load, even in deep tissue layers [58].

One of the major challenges in wound infection is the formation of biofilms. A biofilm is made from a community of bacteria protected by an extremely tough protective matrix, which makes it very difficult for antimicrobials to penetrate [57]. In November 2021, USAMRDC's Military Infectious Diseases Research Program awarded Imbed Biosciences (Madison, WI) nearly $2 million to accelerate development of an advanced version of its MicroLyte Matrix [54]. The new dressing will be designed to specifically target biofilms. MicroLyte Matrix is composed of a resorbable polymer PVA that degrades over time and sloughs off or breaks down through normal metabolic processes. The current dressing also contains ionic and metallic silver to fight bacterial infections and is FDA-cleared for use [61]. The updated version will be extremely thin. In addition to fighting biofilm development, it will be a suitable template for cell growth. The pending DoD award will support a prospective human clinical trial of the new antibiofilm capability of the dressing [54].

Other nanoscale strategies for addressing multidrug-resistant microbes are under development. One emerging area of design is AMPs. These are naturally occurring amino acid chains that can target specific intracellular targets mediated through multiple mechanisms [56]. AMPs electrostatically interact with bacterial cell membranes, leading to physical damage of the bacteria. The action of traditional antibiotics can also be enhanced with the introduction of AMPs. Apart from AMPs, nanoscale formulations of antibiotics, including vancomycin, carbapenems, ciproflaxin, and gentamycin, have been achieved using biomaterials and polymeric NPs to improve localized delivery into damaged tissues [56, 58]. The use of a wide variety of NPs in antimicrobial therapy has blossomed in recent years, and the field is extremely widespread and comprehensive. For additional review and more detailed information on current strategies using nanoscale materials for infection control, see references 56–58.

3.5 BURN CARE

Burns are one of the most traumatic injuries and most complicated to treat due to their significant morbidity and mortality, effects on physical and

psychological health, and impact to almost every major organ system [62]. Burn injuries present several main challenges to medical treatment, namely fluid loss, infection, and pain management. The severity of a burn is related to the extent of involvement of affected tissues based on the location on the body as well as the depth of the burn through tissues (first, second, and third degree). Burn care is extremely important to the DoD. Burns are twice as likely to occur to deployed military members, who are also more likely to die from infection compared with their civilian counterparts [63]. Burns have been of particular concern during recent conflicts. During operations in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF), severe burns constituted 5%–20% of combat-related injuries [63]. Lairet et al. [64] noted that during combat operations in Afghanistan from 2009 to 2011, improvised explosive devices (IEDs) accounted for ~87% of burns. Infection has been discussed as one of the key challenges of burn management, and the use of IEDs in previous conflicts has posed significant challenges to infection control because IED-related burn injuries are more likely to become infected due to contamination with dirt and debris [63]. As mentioned in Section 1, "Introduction," burns remain a significant challenge in CCC and are expected to remain a challenge, if not become a growing one, in emerging conflicts.

Nanotechnology has been and continues to be explored for treating burns primarily in the areas of infection control, wound healing, and tissue engineering. There are six classes or configurations of nanomaterials currently being explored for burn care—nanogels, CS, nanoemulsions, lipid NPs, Ag NPs, and nanofibers.

Nanogels are hydrogels composed of nanoscale particles that have the potential to absorb large amounts of aqueous liquids. They are 3-D structures that work well in wound healing because they mimic the natural structure of the ECM. Nanogels can be loaded with other NPs, like metals and peptides, to diminish bacterial activity and promote tissue growth. Nanogels are biocompatible and promote tissue formation, collagen deposition, neovascularization, and re-epithelialization [55].

As mentioned previously, CS is a natural polymer that has intrinsic healing properties and is used in a wide variety of biomedical applications, especially in wound healing. CS works well for burns due to its ability to enhance fibroblast, macrophage, and polymorphonuclear leukocytes, which aid in tissue repair [65, 66]. Additionally, it is believed that CS's antimicrobial properties stem from four mechanisms:

1. The ability of CS to modify the permeability of bacterial cell membranes.
2. Inhibition of mRNA and protein synthesis.
3. Restricting cell growth through a naturally occurring chelation process.
4. Inhibiting oxygen and nutrients to aerobic bacteria.

CS is also used as a substrate to attach other molecules to, such as growth factors and Ag NPs, which further aid in the healing process of burns. For these reasons, CS is used in a variety of applications for dressing and bandages in would healing and offers substantial promise in treating burn injuries.

Nanoemulsions are formed with oil-based and aqueous solutions that create particles in the range of 20–200 nm [66]. They are thermodynamically stable systems that have an outer hydrophilic coating and an inner hydrophobic core. This structure allows nanoemulsions to be soluble in aqueous media but also carry insoluble agents inside the core. Nanoemulsions can form uninterrupted films over the surfaces of wounds. They have been shown to be highly effective against common bacteria found on burn wounds, including *S. aureus* (including methicillin-resistant Staphylococcus aureus) and *P. aeruginosa* [55, 66]. BlueWillow Biologics (Ann Arbor, MI) is developing a nanoemulsion formulation (NB-201) that is

antimicrobial and potentially inhibits burn wound progression [67]. In studies using a porcine model, NB-201 inhibited both sequestration of neutrophils and production of inflammatory mediators. Data shows that NB-201 limits the progression of partial-thickness burns to full-thickness burns. If true, the company contends that this would "revolutionize treatment for soldiers who experience burn injuries in the field" [63].

Liposomal NPs (liposomes) are typically solid particles made of one or more lipid bilayers that can be formed from a variety of natural and synthetic phospholipids [55, 66]. The structures of liposomes create chambers that can be loaded with a variety of therapeutics agents (e.g., antibiotics, pain medications, genetic materials, growth factors, etc.) which can combine directly with bacterial cell membranes to release drugs (and other items) into the membrane or inside the cell [55]. Histopathological analysis has demonstrated that liposomes can be used to promote the formation of skin appendages and increase the production of collagen [55], which can significantly aid in the remodeling process during burn healing.

Metals, particularly silver, have played a significant role in antimicrobial treatment for centuries, and silver nitrate has been a staple of burn wound treatment for decades [66]. Silver NPs are starting to play a major role in infection control and are often loaded onto other platforms, such as nanogels and CS. Silver is an exceptional broad spectrum antimicrobial agent shown to be effective against a wide range of bacteria, including *S. aureus, P. aeruginosa*, and *E. coli* [55]. One of the best examples of nanosilver in burn care is ACTICOAT (Smith + Nephew – Londin, UK), an Ag NP-based bandage used for treating burns that has been approved for use by the FDA. ACTICOAT is active against over 150 pathogens and begins treating bacteria within 30 minutes of application [68]. Wu et al. [69] demonstrated that a nanosilver burn dressing (Anxin – Zhuhai, China) significantly improved efficiency, wound healing, and healing time and markedly reduced pigmentation fading time, IL-1β, and bacterial positivity rate compared with Sulfadiazine silver cream, a popular pharmaceutical agent used to treat burns. The mechanisms of how Ag NPs may overcome bacterial resistance are thought to be as follows [55, 65]:

- Disturb plasma membrane/cell walls (alter permeability).
- Bind to bacterial DNA preventing replication.
- Bind to ribosomes preventing protein synthesis.
- Inhibit biofilm formation and oxidative stress.

Recent studies showed that nanosilver foam dressings may also achieve pain relief better than other types of Ag NP-based dressings and offer more efficacious results for reepithelization of partial-thickness burns [55].

Nanofibers can be fabricated from natural (e.g., CS) or artificial (e.g., polylactic acid) polymers and typically range from 1 to 100 nm in diameter. Nanofibers that are near the 5-nm range simulate the structure and size of collagen and thus do an excellent job of recreating an environment like the ECM [66], making bioactive nanofibers good candidates for scaffolding that promotes tissue growth and angiogenesis. Many different types of particles (peptides, drugs, etc.) can be loaded onto nanofibers to treat burns, including addressing infection, aesthetics, and pain management. Additionally, nanofiber-based dressings retain moisture in damaged skin, allow for gas exchange, and adsorb wound exudate [55], further addressing significant complications of burn wounds. TuneCoat (Luna – Roanoke, VA) is a nanofiber burn dressing developed with funding support from the U.S. Army Medical Research Acquisition Activity. The dressing is made with electrospun nanofibers that are loaded with drugs to treat pain (lidocaine, fentanyl, and gabapentin) and with silver, to be used as a broad-spectrum antimicrobial [70, 71].

3.6 EMERGING TRENDS

One of the foremost emerging areas in wound care involves monitoring wounds in real-time. This can be a combination of assessing microbial activity, evaluating biomarkers of inflammation, analyzing oxygen concentration in local tissues, and measuring pH and temperature at the wound site. Many types of nanomaterials are being explored for these applications, including Au NPs, carbon-based materials (GR and CNTs), and various types of nanofibers [72–74]. Researchers from the National University of Singapore and Singapore General Hospital developed the VECare platform, a wearable sensor with GR-Au NP electrodes that can detect temperature, pH, bacteria type, and inflammatory factors specific to chronic wounds within 15 minutes [75]. Medical professionals can monitor the status of a wound via a mobile app. A more simplistic but effective application for monitoring wound status, especially for the presence of infection, is a flexible nanofiber membrane developed by ParaNano Wound Care (Oklahoma City, OK) [76]. The Nanosheet Biosensor changes color in the presence of infection (green indicates the wound is infected) and provides a method of continuous wound monitoring between clinician visits. While some excellent wound monitoring and sensing technologies are under development, most of what is available appears to focus on chronic wound management and is not currently suitable for the acute wound care and prolonged field care that the DoD requires.

The field also is leaning toward a few other emerging trends. There appears to be a shift from developing purely passive membranes that cover a wound to more active dressings that interact with and respond to a wound in near real-time. Additionally, R&D supports the use of more novel materials to create dressings, including polyurethane foam films and GR [48] and an increased use of natural and biomaterials, indicating a shift toward working with "green" and more sustainable materials. Further research involves using nanocarriers to deliver therapeutic agents for tissue engineering via siRNA and other gene therapy tools, as well as an emerging interest involving plasmonics and photonics with nanomaterials for wound healing. More novel sensing research is also underway; however, most of these technologies currently exist at extremely low TRLs or still reside in basic research stages.

3.7 CHALLENGES

One of the key challenges to using nanomaterials in wound care is commercialization. Most of the R&D in this area never makes it into commercial markets. This is primarily due to a typical "valley of death" problem in innovation. However, some of it relates to a lack of focus on the translational aspect of wound dressings, especially for what it takes to be successful in combat situations. In some cases, it is entirely too easy to take a dressing to market due to the relatively easy nature of the FDA-clearance process for these devices. Overall, the market becomes too saturated with wound care products that only offer incremental change and may not have much practical purpose for DoD needs. There is also a health and safety aspect of materials research that is still underdeveloped in many ways. Toxicity assessments are not readily available for many nanomaterials, especially long-term biokinetic modelling for biomaterials like CS. Metal NPs have been more thoroughly investigated for toxic effects, but other emerging ones like GR still have a way to go regarding the availability of effective safety information. One way to address this is to test materials early and get good information on what the FDA is likely to approve [77]. All materials, especially those used in biomedical applications, must go through regulatory processes. However, there needs to be a balance between developing the material and developing the product [77]. Although nanomaterial R&D has made some considerable and consistent progress in the wound care market, cost and scalability remain significant challenges to successful commercialization. Currently, most bandages and dressings reside at a TRL of 5. There have been very little cost benefits shown and, in some cases, the nanomaterial coatings cost more than the bandages [78].

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SECTION 04 TISSUE REGENERATION AND ENGINEERING

4.1 INTRODUCTION

Since September 11, 2001, an average of 60,000 Warfighters have been injured during combat in the Afghanistan and Iraq wars, with IEDs making up over 75% (45,000) of all combat and polytraumatic injuries [79]. Of those, 40% sustained fractures, traumatic amputation, and spine injuries. These traumatic injuries have led to long-term disabilities, lost duty days, and economic burdens. Statistically, 64% of combat-related bone and joint injuries in Warfighters have caused permanent disabilities.

A significant health concern related to IEDs for the military is maxillofacial injuries. In a retrospective chart review of the Expeditionary Medical Encounter Database from 2004 to 2010, the prevalence of combat-related maxillofacial injuries was 22.7% (1,345 Warfighters) [80]. Among facial burns and midface traumas, 65.7% of injuries resulted from IEDs, and 64% of the study's participants also sustained a TBI. Most facial fractures (55%) were open, facial fractures, and 64.2% of the injured underwent surgical repair. These blast-induced fractures influence mobility and quality of life and are highly associated with infection and improper healing if not treated immediately.

Retired Army Colonel Philip J. Belmont, MD, stated that military personnel "who were engaged in combat operations over a 15-month period were three times more likely to suffer non-combat musculoskeletal injuries than combat musculoskeletal injuries" [79]. Military service members who sustained orthopedic injuries on the battlefield required immense treatment and long recovery times upon return. According to Koltun et al. [81], stress fractures are musculoskeletal injury concerns with an "incidence rate of 5.69 per 1,000 person-years" which can lead to a stress reaction and complete bone fractures.

Tissue engineering and regeneration medicine focuses on developing a replacement for damaged or diseased tissue by integrating biological properties. There are three components for a successful design—scaffolds, stem cells, and growth factors. The nanocomposite scaffold is a structure embedded with latent stem cells for differentiation to support cell growth and tissue formation. Growth factors "regulate the process of differentiation and proliferation" [82]. Because bone tissue is naturally a biological nanocomposite, it is realistic that researchers and innovators ingrate nanotechnology to mimic the surface in scaffold to encourage new cells to adhere. Technology centered around NPs provides advanced healing properties, such as anti-inflammatory, antibacterial stabilization through the hydrogel, and 3-D bone printing. These innovations decrease clinical demand, lengthily multisurgeries, and recovery times while enhancing skeletal healing, bone density, and quality of life for the Warfighter.

4.2 BONE TISSUE ENGINEERING

A bone is "a mineralized connective tissue that consists of three types of cells (osteoclast, osteoblast, and osteocyte) as well as a biphasic

extracellular matrix (the percentage of mineral part to organic content is approximately 7:3)" [83]. In bone tissue, cells and collagen fibers (Col-I) build up the skeleton from which minerals are deposited, which is controlled by growth factors. The minerals and skeleton together build the bone's strength and density. Given an adequate blood supply, "bone tissue has a certain regenerative potential and can renew itself via constantly undergoing bone remodeling in order to adapt to the ever-changing body load and maintain the necessary mechanical strength" [83]. However, not all injuries can heal on their own. Traditionally, severe injuries require bone repair through a method called bone grafting.

Bone grafting is used because it replaces the missing bone and helps promote new bone growth. There are several methods of obtaining materials for bone grafting, which are used from autogenous bone, allogeneic bone, xenogeneic bone, and artificial scaffolds. Autogenous bone grafts are taken from the patient's body, typically from the chin, jaw, lower leg bone, hip, or skull [84]. "Due to excellent bone conduction, osteoinduction, osteogenesis, available source, ideal biocompatibility, and 3-D structures, autogenous bone has been regarded as the gold standard in bone defect repair materials" [85].

Allogenic bone grafts are also human bones; however, they are harvested from donors, typically a cadaver. The allogenic bone graft is then processed using a freeze-dry method to extract the water via a vacuum. Unlike autogenous bone grafts, allogenic bone cannot promote new bone growth. Instead, the allogenic bone graft is used as a framework or scaffold for the patients existing bone to grow and fill the area.

Xenogeneic bone grafts are harvested from a different species, typically a cow. "The bone is processed at very high temperatures to avoid the potential for immune rejection and contamination" [84]. Like allogenic bone grafts, xenogeneic bone grafts are used as a framework or scaffold for the patient's existing bone to grow and fill the area.

4.3 APPLICATION OF NANOMATERIALS IN BONE TISSUE ENGINEERING

Due to recent advancements in regenerative medicine and tissue engineering, the application of nanotechnology to regenerative medicine has increased. Using engineered bone tissue has been viewed as an alternative to conventional bone grafts due to its limitless supply and no disease transmission. Longstanding research has focused on using metals, bioceramics, and polymeric components as regenerative biomaterials for bone. Recently, research for bone repair has shifted toward using hydrogels, glass-based nanocomposite, nanofiber scaffolds, the application of nanotechnology in targeted drug delivery, and 3-D printed composite scaffolds.

There are three main properties of nanostructured biomaterials that are important to keep in mind to ensure successful bone regeneration (Figure 4-1). They are as follows:

1. **Mechanical Properties** – bone formation response can also be changed by altering the mechanical environment.
2. **Biocompatibility** – the biocompatibility of biomaterials plays a significant role in their performance for bone healing.
3. **Osteoinductivity** – induces new bone formation.

4.3.1 Bone Tissue Engineering Scaffolds

Bone is a naturally occurring micro- and nanostructured composite, which makes it a natural fit to use nanomaterials in its reconstruction. Lyons et al. [87] reported that nanostructured biomaterials often prove superior compared to larger scale materials when used to enhance bone tissue regeneration. There are four elements to consider when using nanostructured materials in bone regeneration—composition, physical stimuli, architecture, and biochemical cues.

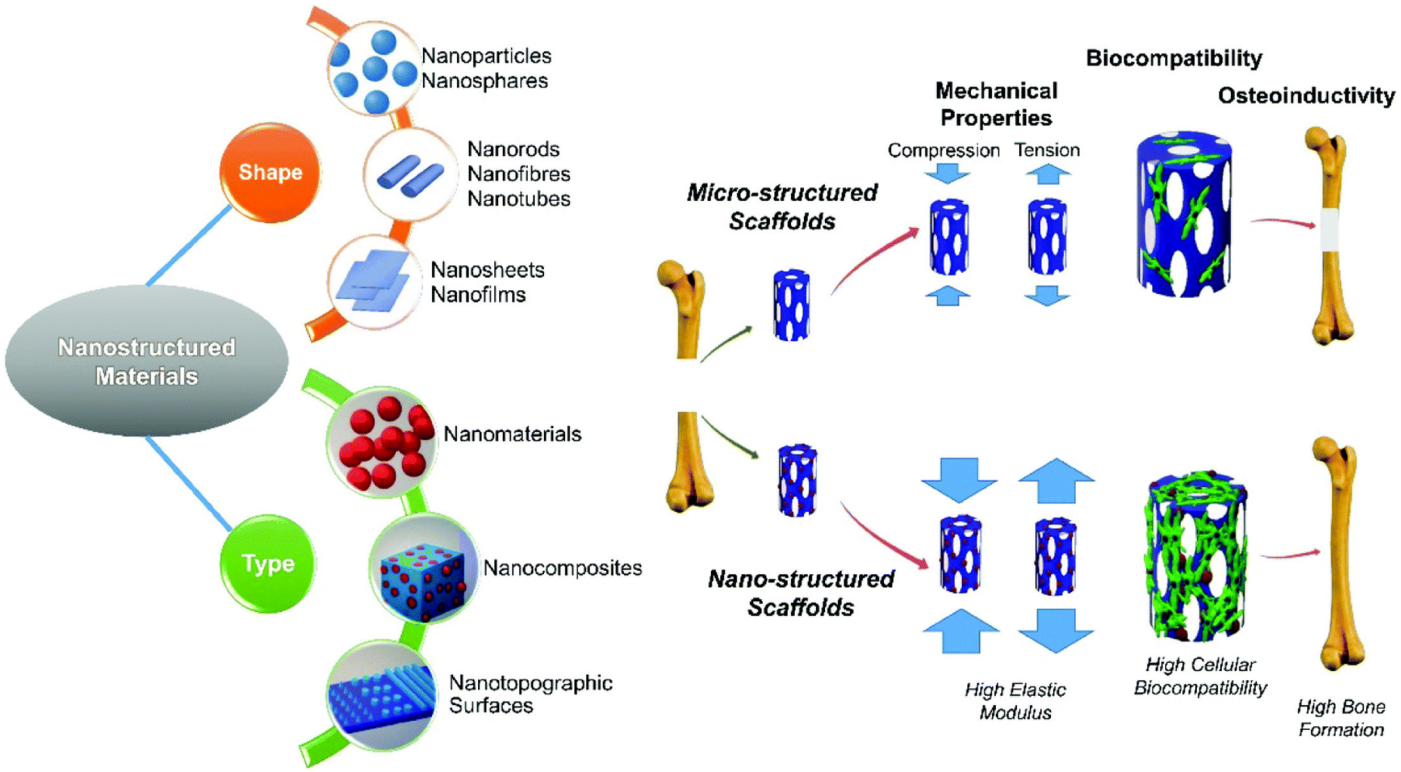

Figure 4-1. Nanostructured Materials for Bone Engineering *(Source: Hajiali [86]).*

One of the key methods of meeting these design elements while supporting bone regeneration is to use scaffolds, which are 3-D porous structures that can stimulate the growth of new bone tissue [82]. Some of the goals in designing scaffolds are to obtain implants capable of replacing native tissues, support the tissue regeneration process, and minimize complications. Successful scaffolds must meet the following five basic criteria [82]:

1. Have a 3-D shape with high porosity.
2. Are biocompatible.
3. Have a controlled rate of degradation and resorption.
4. Contain surface chemistry that allows cell binding, proliferation, and differentiation and be like tissues at the site of implantation.
5. Provide sufficient mechanical strength and support.

Scaffolds can be designed and constructed using a multitude of nanomaterials and nanostructures. Some of the most researched ones are as follows:

- **Silica** – Silica NPs can stimulate bone cell growth and deliver bioactive molecules, such as dexamethasone, growth factors, vitamins, and mineral ions [82].
- **Carbon-Based Nanomaterials** – CNTs and graphene oxide (GO) have been explored for their potential to influence bone regeneration, effective cell proliferation, and osteogenic differentiation [88].
- **Bioceramics** – Naturally occurring ceramic materials can promote osteochemical formation, osteogenesis, and vascularization. Common materials include calcium phosphate (CaP), nano-hydroxyapatite (n-HA), and bioactive glass [82].
- **Nanopolymeric Scaffolds** – These nano-structures are easy to work with and adaptable, have good biocompatibility, resorb through natural metabolic pathways, and improve osteoconductive and osteointegration. Common materials include poly-L-lactic acid, poly(lactide-co-glycolide) (PLGA), polycaprolactone (PCL), silk, and CS [82].

- **Hydrogels** – Hydrogels are materials that absorb water and allow cells to adhere, multiply, and differentiate. They work well in tissue regeneration due to their structural similarities to the ECM. Moreover, hydrogels create a porous framework that allows cell transplantation and proliferation. One advantage of hydrogels is their ability to take the desired shape of bone defects. Commonly used materials include natural materials like agarose, alginate, and gelatin and synthetic materials such as PVA [82].
- **Nanofibers** – Nanofibers are structures typically made through electrospinning (see Section 3, "Wound Management and Infection"). They are more suitable for scaffold components than other types and shapes of nanomaterials. Along with hydrogels, they can also be used to create an environment that more closely resembles the ECM. Nanofibers possess advantages, such as high porosity and high surface-to-volume ratio, helping to induce favorable behaviors (adhesion, proliferation, and differentiation) [89].

In addition to structural materials to construct scaffolds, several types of nanomaterials are used to enhance the properties of scaffolds and improve bone regeneration success.

- **Metals and Oxides** – Metal NPs have several characteristics that make them good candidates for bone healing, including good biomechanics and thermochemistry, stability and optical behavior, reduced toxicity and good biocompatibility, osteogenic potential, cellular development modulation, antimicrobial effects, and anticancer effects [90]. To this end, several types of metal NPs have been explored for their use in bone healing and regeneration.
  - **Magnesium (Mg)** and alloys demonstrate suitable biocompatibility and high mechanical strength but have low corrosion resistance in physiological environments. nHA coatings can be used to decrease the degradation of Mg and improve mechanical strength of implants. Mg is often used in bone healing because it promotes osteoinductivity and osteoconductivity [88].
  - **Titanium** is widely used as a surgical material and in implants due to its high biocompatibility. It does have poor antibacterial properties, but these can be enhanced using surface coatings made with Ag, nHA, CS, or other materials [88].
  - **Gold (Au)** is a commonly used NP in medicine due to its biocompatibility and relative bioinertness. It is often researched for bone regeneration due to its high surface functionalization potential. Additionally, Au NPs have intrinsic osteogenic effects, causing stem cells to undergo osteogenic differentiation. Because gold also retains antimicrobial and anticancer properties, Au NPs are often embedded into composites for bone repair and regeneration [90].
  - **Silver (Ag) NPs** stimulate osteogenesis and inhibit osteoclastogenesis. Ag is commonly used to induce antimicrobial effects in clinically used devices while stimulating osteogenic activity. Modifying surface coatings with Ag NPs is a solid strategy to enhance bioactivity. Like Au NPs, Ag NPs are often embedded within polymer coatings for antibacterial, osteogenic, and bone-forming potential on implants [90].
  - **Copper** is an essential element vital to normal function of bones, blood vessels, and nerves. It often plays a role in wound healing and immunity due to its antibacterial properties. Copper plays a very beneficial role in bone metabolism and can enhance mineralization, osteogenesis, angiogenesis, and modulated osteoclastogenesis [90].
- **Liposomes** – Nanostructures with hollow cores that can be imported into scaffold systems and used to deliver therapeutics during the tissue regeneration process [82].

- **DNA Nanostructures** – Plasmid DNA and/or tetrahedral DNA nanostructures that can be used to enhance scaffolds or deliver biological agents like growth factors and genes to injured sites [91].

4.3.1.1 Three-Dimensional Printing of Bone Regeneration Materials

Over the past 10 years, vast improvements in bone regeneration science have opened another frontier—the application of AM techniques to the fabrication of bone scaffolds and other tissue structures. Most common among AM techniques is 3-D printing, or the process of creating a 3-D object from a digital design file. The foundational methods of 3-D printing include material deposition (via nozzle), selective sintering (powder fusion/solidification), sterolithography (ultraviolet [UV]-based photopolymerization), jet prototyping (binder injected into powder), and laminated manufacturing (cutting and gluing of layers) [92]. Currently, the number of proven 3-D printing techniques has grown so large that no comprehensive classification system for them exists, especially since some methods, like polyjet modeling, combine principles of multiple approaches (stereolithography and injection). Because some newer 3-D printing techniques also combine both additive and *subtractive* fabrication methods, the more appropriate term for the field is *advanced manufacturing* [92].

Originally viewed as most beneficial for rapid prototyping and as an aid to digital design, AM techniques have since found their way into full production schemes. Moreover, the expansion of AM processes has occurred in tandem with growth in the portfolio of materials suitable for use in AM, including metals, ceramics, composites, and both natural and synthetic polymers. (3-D bioprinting, or the controlled placement of living cells into a desired 3-D pattern, is also a proven but still nascent technique [93].) For 3-D printing of bone scaffolds, candidate AM materials must possess the same qualities as traditional scaffold media, including biocompatibility, pore interconnectivity, porosity, and other specific mechanical properties [94]. However, the characteristics of a particular bone injury may dictate the use of a particular 3-D printing process and/or material. For example, selective laser sintering can print scaffolds from biocompatible and biodegradable polymers with high compression strengths, but the lasering process can often result in lower porosity than other methods—a critical quality for bone scaffolds [94].

The benefits of using AM and 3-D printing for bone scaffold fabrication are many. It allows for the design of geometrically complex parts with internal channels and variations in thickness, porosity, and mechanical properties; it also allows for precise customization of a part to fit an irregular void. The ability of some AM processes to use multiple materials also enables new functionalities [92]. Custom-designed, patient-specific AM bone scaffolds have been shown to improve healing outcomes and physical appearance relative to traditional methods [174]. Just as relevant for CCC is AM's concomitant reduction in fabrication times and production costs—future bone scaffolds may be easily made in remote medical treatment facilities.

The use of nanomaterials in AM bone scaffolds has already shown great promise, going well beyond the straightforward integration of nanomaterials (like n-HA/PCL) into the raw materials used by a 3-D printer [174]. One prominent thrust of work involves the 3-D printing of a scaffold or structure with a porosity calibrated to fit a nanoenabled hydrogel or other nanomaterial coating like nanoceramics or single- and multiwalled carbon nanotubes. Kankala et al. [95] 3-D printed a bone scaffold via extrusion using PLGA and then coated it with an nHA gelatin. While PLGA is one of the three most preferred polymers for 3-D printing of bone structures, it can display less-than-optimal mechanical properties and poor hydrophilicity when used alone. By doping the structure with the n-HA gel, the scaffold achieved good hydrophilicity, biocompatibility, and significantly promoted the proliferation of osteoblasts.

Qiao et al. [96] similarly produced a porous titanium alloy (Ti6Al4V pTi) bone scaffold via electron beam melting to fit a defect in an infectious bone. Displaying good macroscopic morphology and microscopic structure, the pTi scaffold was then coated with a multiple-mixture hydrogel that included Ag NPs for antimicrobial performance. The completed implant demonstrated effective antibacterial action and successfully promoted bone regeneration and osseointegration. A similar study combined copper-loaded-ZIF-8 NPs (Cu(I)@ZIF-8) and a PLGA, extrusion-based scaffold to promote regeneration of an infected bone injury [97]. Composite nanomaterial scaffolds like these combine the excellent bone conductivity and biocompatibility qualities of the nanoenabled coating with the ease and flexibility of design provided by 3-D printing techniques, including the precise control of porosity across the structure. Evidence also suggests that the application of nanobased coatings reduces the importance of precisely matching the stiffness and strength of the 3-D printed scaffold to the surrounding bone, opening new avenues of research around flexible and hyperelastic bone grafts and scaffolds [86].

An emerging trend in the use of nanoscience within the 3-D printing of solid bone regeneration materials is the development of bioprinting—using a living-cell-infused biopolymer gel or "bio-ink." Multiple AM methods are available for bio-inks, including extrusion, inkjet-based printing, and stereolithography. The contribution provided here by including nanomaterials in the bio-ink is critical. As Cheng et al. wrote, "Nanomaterial-based bio-inks provide easier processability, higher stiffness and degradation, and functional ability due to their physical properties and nanoscale features to promote cell and bone growth, reduce infection rates, and enhance tissue regeneration" [98]. Zhai et al. developed a hybrid bio-ink using hydrogen bonding monomers and nanoclay printed into a composite scaffold strengthened via UV light illumination of the printed prehydrogel [99]. The cross-linking of the nanoclay, combined with the hydrogen bonding, delivered strong mechanical performance and facilitated the regeneration of new bone when tested in tibia defects of rats. The current landmark work in this area comes from Alcala-Orozco et al., who developed a multifunctional hybrid nanocomposite biomaterial ink made from Mg hydroxide NPs and PCL thermoplastic [100]. Displaying high structural stability and biological functionality, this bio-ink was printed at high fidelity to original designs. Alcala-Orozco et al. further delivered a proof of concept using Mg-PCL in combination with a bone-specific bio-ink (Strontium-carbonate [Sr] NPs and low concentration [5 w/v%] gelatin-methacryloyl – Sr-GelMA) via coordinated extrusion to produce hybrid bone constructs. This process demonstrated that the use of nanocomposite bio-ink, when optimized for extrusion-based 3-D printing of regenerative bone scaffolds, delivered enhanced mechanical stability as well as bone-related bioactivity for promoting regeneration [88]. As Qiao et al. described their work in the *Journal of Nanobiotechnology*, this "recently developed hybrid biofabrication approach allows the fabrication of bone draft with enhanced mechanical properties … and improved osteogenesis … which unravels the ultimate potential of 3-D printing in bone engineering" [89].

4.3.1.2 NuShores Biosciences

NuShores Biosciences, LLC, was founded in 2014 through discoveries made at the University of Arkansas at Little Rock with grant research funds of over $12 million. Their technologies focus on a "commercialized patented bone and tissue regeneration" solution called NuCress, a scaffold platform [101]. NuCress is an implantable, nanomaterial-based bone scaffold device that has been tested on large and small animals (goats and mice) for large segmental bone breaks (>2.5 cm) and shows healing factors with no infections, inflammation, rejection, and adverse bone response. A record-breaking accomplishment was the repair of an 8.5-cm goat tibia [102]. The components used for scaffold in bone generation eliminate special instruments, as the implant swells

to lock into place in the gap left by the fracture while delivering stability as the bone heals naturally.

NuShores' research shows the effective and safe use of alternative to bone regeneration in the craniomaxillofacial, dental, limbs, or spine [101]. Nanoscaffolds are used in tissue engineering; they supply hormones or stem cells for bone growth and antibacterial drugs to fight infections [103]. The benefits of nanotechnology are the targeted drug carriers, which are minimally invasive, and the biomimetic characteristics that contribute to limiting complications. Common NPs used for bone tissue engineering and bone disease are "liposome, polymeric nanoparticles, dendrimer, silica nanoparticles, carbon nanotubes, quantum dots, gold and bioceramic nanoparticles" [82]. NPs play an important role in tissue regeneration due to their size for model scaffold and mechanical properties. Nanotechnology permits the scaffold to be manipulated in shape and design while providing a stable shelf life.

"NuShores was founded with DoD goals in mind," said Dr. Alex Biris, Director of the UA Little Rock Center for Integrative Nanotechnology Sciences, leader of the DoD grant, and cofounder of NuShores [101]. Approval for the manufacturing of Generation 1 NuCress bone void filler scaffold products came after a $5.6 million grant by the DoD in 2019. Later in 2020, NuShores Biosciences, LLC, "won a 3-year, $2.8M contract from the Medical Technology Enterprise Consortium (MTEC), a 501(c)(3) biomedical technology consortium associated with the U.S. Army Medical Research and Development Command" [104]. The new contract creates opportunities for further potential to reduce human error in real-time situations and expand future investments with the concept of a factory-in-a-box (FIAB) feature. FIAB creates the opportunity for manufacturing a product in an easily transportable container that requires minimal utility and environmental support. Smart manufacturing can promote the clinical use of bone regeneration scaffolds in the hand of surgeons and the DoD. Subsequently, in 2021, NuShores received a three-year $2.9 million commercialization readiness grant from the National Institute of Dental and Craniofacial Research. The funding empowers NuShores to achieve the required FDA design controls processes for the NuCress scaffold for dental indications, moving one step closer to the human market [105]. The company is expected to apply for FDA approval of NuCress in 2022.

NuCress bone void filler is a nanocomposite bone scaffold that provides architectural support for cell growth in tissue engineering. The highly porous scaffold is 3-D printed for a variety of shapes and sizes to accommodate bone defects, with the greatest benefit to generate neovascularization in as early as three weeks [102]. The natural healing process depends on osteoinduction, where undifferentiated or stem cells stimulate the development of preosteoblast activated by the trauma [106]. Consequently, osteoconduction uses the differentiated osteogenic cells that develop into osteoblasts for bone formation and mineralization. NuCress, an osteoconductive material commonly seen in bone implants, allows bone cells (osteoblast and osteoclast) to grow, attach, and migrate across the scaffold, slowly replacing the gap with new natural bone growth triggered by the fracture [102]. Due to its biocompatibility, the filler resorbs as it is replaced with natural bone, restoring the quality of life.

NuShores' scaffold has been used successfully in several animal studies. In 2021, at The University of Tennessee College of Veterinary Medicine, the scaffold was used to treat 2-cm metacarpal bone defects in five horses. The 3-D printed porous scaffold was composed of polyurethane (PU), HA, and decellularized bone particles (DBPs) [106]. PU has pliable properties to maintain the integrity of the structure. Implanted scaffolds are configured to be biocompatible and degradable. As the new bone tissue replaces the void, a PU platform meets these characteristics. n-HA stimulates osteoblastic cell adhesion and proliferation while providing calcium-containing minerals and alters the surface

energy to manage protein adsorption and inhibit the inflammatory response. The 60-day study evaluated the effectiveness of the scaffold as a bone filler for tissue regeneration when compared to natural healing with an empty void [107]. Results through radiography presented a statistically significant difference between the scaffolds and the controlled, unfilled defect ($p = 0.006$). The scaffold showed substantial bone filling ($67.42\% \pm 26.7\%$) when compared with controlled defects ($35.88\% \pm 32.7\%$). The average density immediately after surgery revealed the scaffold was 449.8 ± 137.1 Hounsfield Units (HU), and the controlled defect was 83.20 ± 133.4 HU ($P = .003$). When revaluated at 60 days, the density significantly increased in the scaffold to 807.80 ± 129.6 HU, and the controlled defect was 464.80 ± 81.3 HU ($P = 0.004$). The study supported early bone formation in the scaffold influenced by NP and DBP to encourage osteoblastic proliferation. The scaffold provided various porosities with "small pores (<150 μm) that facilitate early cellular migration with micro vascularization, and large pores (>300 μm) facilitate larger blood vessel formation, resulting in vascularization of the graft and sustained tissue formation" [107]. Evidence showed strong osseointegration, with on-growth bone formation on the outer surface and in-growth within the implant, without an interlayer of fibrous tissue between the implant bone's surface.

4.3.1.3 Mesenchymal Stem Cells

Another study conducted at The University of Tennessee College of Veterinary Medicine received partial funding from USAMRMC to evaluate human mesenchymal stem cells in conjunction with NuShores' scaffold technology [108]. Human mesenchymal stem cells (hMSCs) derived from human adipose tissue were used to demonstrate the mechanisms of biomaterial through transcriptomic and metabolomic analyses. These cells have the potential to differentiate an important reparative element. In the study, the adipose-derived hMSCs (adhMSCs) were embedded into the nanocomposite scaffold, creating an osteogenic platform. The 3-D nanocomposite scaffold proved to be cytocompatible, demonstrating significant upregulation of genes linked to osteogenesis and osteobiologic characteristics. The transcriptomic data expressed positive support for the adhMSCs scaffold, and the detection of metabolites like ascorbate was strongly associated with osteogenesis.

The University of Pittsburgh's Center for Craniofacial Regeneration (CCR) has partnered with McGowan Institute for Regenerative Medicine and the USAISR on a three-year, $2.1 million grant to accelerate bone healing [109]. Bone tissue engineering has been an interest to the DoD due to the burden that military personnel experience with traumatic bone injuries. An average of 20% of military personnel sustain an extremity bone fracture in combat. The accelerated bone-healing nanotechnology created through this grant is an off-the-shelf biological device offered at the POC. The device is loaded with minimally manipulated autologous MSCs delivered with a hydrogel. NPs provide extended drug release and control immune response while promoting healing. The porous hydrogel-infused scaffold contributes to the mechanical structure support in large bone defects. The POC actions allow first responders to utilize the injectable device to stabilize bone fragments, prevent fibrous tissue ingrowth, and promote bone formation. This ideal therapy would minimize severe morbidity while enhancing treatment options to reduce cost and recovery time for military personnel.

Researchers from Texas A&M University leveraged the potential of stem cells to assist in bone regeneration [110]. Previous research has shown that two-dimensional (2-D) covalent organic framework (COF) NPs manage the differentiation of hMSCs into bone tissue. A discovered limitation was the processing of COF into nanosized material and the questionable stability. Dr. Ashilesh K. Gaharwar, associate professor and a fellow of the American Institute for Medical and Biological Engineering, and his team strengthened the

hydrolytic stability of COFs by incorporating amphiphilic polymers. These macromolecular polymers exhibited hydrophobic and hydrophilic characteristics, further empowering the biomedical application of NP. This signified that the NP properties enhanced and prolonged drug delivery, as demonstrated with the osteo-inducing drug dexamethasone, which is associated with positive bone formation.

The U.S. Army Research Office partially funded research conducted by New York University (NYU) and the New York Stem Cell Foundation Research Institute that used a process known as biothermal scanning probe lithography (bio-tSPL) to sculpt a biocompatible material at the nanoscale. These bone replicas were then used to support the growth of bone stem cells derived from the patient [111].

As previously mentioned, Au is often used in bone formation to enhance certain functionalities. Researchers at the University of Southampton have explored a method to identify viable bone stems cells by using Au NPs coated with oligonucleotides to detect mRNA in bone stem cells [112]. This process creates a fluorescence that reveals which cells to target in bone marrow for harvesting.

Researchers at McGill University in Montreal created a bone scaffold using an emulsion added to GO [113]. The mixture was then frozen several times, creating multisized pores throughout the material. When stem cells were added to this scaffold, the structure promoted their transformation into osteoblasts. This was the first time that a synchrotron was used to see the structure of GO scaffolds.

4.3.1.4 Veterans Affairs (VA): The BioBone Project

In 2020, "the VA performed nearly 400 mandibles (mouth/jaw) tumor resection, craniofacial reconstruction and bone harvesting surgeries for cancer patients and Veterans with chronic facial injuries or infections that could have benefited from 3-D printing innovations—a market predicted to be $4 billion in the U.S. by 2028" [114]. The VA's Office of Research and Development and VA Ventures joined the partnership with the Biofabrication Community of Science to create a network of researchers, engineers, and innovators to develop best-of-class care for veterans. The Veterans Health Administration (VHA) 3-D Printing Network has grown its pipeline to include 75 AM sites since 2021. The VHA is leading the field in AM with its support to provide personalized applications to address the unique needs of patients. The first three patient-matched products in the pipeline are surgical anatomic guides for maxilla or mandible (jawbone), radiotherapy boluses for facial malignancies, and presurgical 3-D visualization of the patient for cardiac procedures [114].

The VA's Puget Sound's VA Venture was established on June 25, 2020, as an innovation incubator to guide healthcare innovations toward long-term solutions [115]. The director of the VA Ventures, Dr. Beth Ripley, is focused on growing the biofabrication field through the BioBones Project, which involves using a fully biodegradable composite of resorbable phosphate fibers and polyester as a bone graft alternative. This nonsynthetic, vascularized implantable bone was designed and developed as a bone substitute regeneration treatment for bone tissue repair through a 3-D, bioprintable structure. Previous autografts, known as bone harvesting, are invasive and require using bone from another part of the body and then grafting it to replace the damaged tissue. These surgeries can last an average of 10 hours or more. The future of BioBone creates 3-D printing of the artificial bone grafts from each patient's blood cells. This customized anatomy match maximizes bone healing ability and health outcomes. The 3-D printing improves communication and provides a personalized health care solution tailored to each patient. Advanced Solutions Life Sciences has partnered with VA Ventures by designing the BioAssemblyBot for 3-D printing. It is anticipated that this technology will be in VA hospitals within the next three to five years,

allowing bone grafts to be created on-site, reducing wait times and improving success rates [114]. The future of the BioBone Project is transitioning from long bone repair to irregular bone repair, with 3-D printing for bone regeneration.

A team of researchers at Queens Medical Centre in Nottingham developed a similar mechanical property to the BioBones' composite, which slowly degrades as native bone replaces it [116]. These characteristics eliminate additional surgeries to remove pins and plates with customized surgery bone repair. The structure allows the polyester to be molded and reformed at low temperatures to fit specific patient needs in surgery. Preclinical trials have been successful, as initial applications include low load-bearing bones, orbital floor, cranial plates, spinal fusion, jaw/cheek plates, and finger/toe plates.

4.4 CHALLENGES AND CONCLUSION

Bone tissue regeneration has made considerable leaps forward in recent years, partially due to the successful integration of nanotechnology; however, challenges remain in this field. Hajiali et al. [86] neatly outlined key challenges in the following three areas:

1. Mechanical Properties
    - No growth in nonporous structures.
    - Lack of knowledge of nanoeffect.
    - Interaction between cells and environment during bone growth.
2. Biocompatibility
    - Cytotoxicity of nanomaterials.
    - Assays do not elucidate mechanisms of cell death.
    - In vitro results do not translate to in vivo results.
3. Osteoinductivity
    - Systemic toxicity of nanomaterials.
    - Competition between osteogenesis and angiogenesis.
    - Difficulties in clinical translation (animal to human trials).

Historically, bone tissue grafts use autografts (harvesting a patient's bone), allografts (cadaveric donor bone), or synthetic grafts. Natural or synthetic bone grafts have limitations, coupled with lengthy multisurgical procedures and long recovery times. One of the leading causes of implant failures is an infection—the nanotechnology scaffold is designed to be biomimicking, reducing the risk of infection [82]. Another limitation in bone tissue regenerations is donor availability and the mismatch of cell proliferation and differentiation. NPs have excellent mechanical properties for osteointegration, osteoconduction, and osteoinduction. The application of nanoscaffolds during the healing process to replace native bone increases bone integration while minimizing complications.

SECTION 05 NEUROTRAUMA AND PAIN CONTROL

5.1 INTRODUCTION

The DoD has been interested in nanotechnology for decades, as combining nano in the medical field for military use has been an increasingly attractive topic. According to the DoD's Fiscal Year Budget estimates in the year 2021, there were $45.3 million allocated to fund projects in programs of living systems' response to biological or chemical agents to support detection, diagnostics, protection, medical treatment, and promote research, including nanotechnology and nanoscale science [117]. The physical science aspect of the budget aims to fund nanotechnology that could be used for defensive (protection) capabilities, enhancing Warfighter performance and safety through research in nanomechanical resonance sensing and increasing medical effectiveness. In addition, this portion funds animal research to investigate nanocatalyst synthesis in a more cost-effective approach.

A key research question is how nanotechnology can be manufactured for upscale use and provide effective, long-lasting, safe, high drug load, and lightweight technologies to protect Warfighters. The U.S. Army Institute of Surgical Research (USAISR) set its goals to optimize combat casualty care. A branch of the Institute is the Pain and Sensory Trauma Care (CRT5), and its mission is to link new applications for treating traumatic induced pain as well as research to repair and store the sensory system from injuries sustained by Warfighters. The paradigm shift incorporates multimodal application for battlefield pain control without physiological or cognitive impairment and nonopioid drug alternatives at the POC. Blood-based biomarkers and novel drugs for acute pain management are facilitating the development of future protocols within USAISR [118].

The Neurotrauma and Traumatic Brain Injury Portfolio under the Combat Casualty Care Research Program strives to improve the military diagnostic and treatment for the spectrum of TBI. Multidomain operations highlights moderate and severe TBI as well as polytrauma to field and deployment settings. The issues that are requiring more attention for research are fielding POI intervention to control secondary injury to the brain, rapid accurate diagnosis and monitoring TBI field care, developing sensors and monitoring devices for the field environment, and developing life-saving and stabilizing interventions for TBI. When evaluating active-duty TBI cases, evidence shows that the Army represents more than 54% of the overall cases [119]. Therefore, Army research partners are vital to the future protection of the Warfighter. The partnership with the Institute for Soldier Nanotechnologies allows collaboration in research focused on solving these issues with a new approach to nanotechnology. Nanomedicine integrated into the military provides a high drug load with specific cell targeting technologies to boost healing, a drug delivery system for the blood-brain-barrier (BBB), and a nonopioid pain management routine for longer lasting effects for field operations. The nanomaterials can be utilized

for diagnostic biosensors, blast-pressure monitors, and protection strategies in smart helmets. Lastly, NPs for effective blood-based biomarkers, therapeutic interventions, and antioxidant and anti-inflammatory defense are critical for TBI.

5.2 TRAUMATIC BRAIN INJURY (TBI)

5.2.1 Overview

TBI is one of the leading causes of death and disability in the United States, affecting nearly two million people per year [120]. The clinical spectrum of TBI ranges in severity based on how responsive the individual is after injury and the length of losing consciousness and memory loss or disorientation. The severity of TBI ranges from mild to moderate (brief disorientation or loss of consciousness), such as an asymptomatic, subconcussive blow, to severe (extended loss of consciousness or a penetrating brain injury), often leading to significant brain damage. Depending on its severity, TBI can be accompanied by long-term disabilities [121]. Clinical symptoms could include headaches, dizziness, motor impairment, fatigue, irritability, memory, and cognitive problems.

The primary injury through a force or blast is then followed by a secondary injury—causing physiological change to the brain. Treatment targets the accumulation of reactive nitrogen species and reactive oxygen species (ROS), glutamate toxicity, mitochondrial dysfunction, and neuroinflammation. TBI causes a positive feedback loop where the primary physical damage to cells follows biochemical disruption and "damage-associated molecular patterns (DAMPs), leading to further cell death and the release of additional biochemical derangements and DAMPs" [122]. A wide-ranging focus in TBI research is the relationship between injury and neurodegeneration.

Overpressure (OP) is the pressure caused by a shock wave that is above atmospheric pressure; exposure is caused by explosive devices or charges. Military personnel who engage in training and combat roles are at risk for high exposure to OP. The Defense Automated Neurobehavioral Assessment is a neurocognitive and psychological assessment tool developed by the DoD. The assessment measures the change in performance over TBI-related symptoms and operates in combat deployment settings. A more recent diagnosis option being explored is blood biomarkers. Central nervous system (CNS) proteins, such as neurofilament light chain (Nf-L), tau, glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), precursors of amyloid protein, and amyloid-beta (Aβ) peptides are biomarkers associated with TBI. GFAP and UCH-L1 are not typically present in individuals with mild TBI (mTBI) unless hemorrhage or intracranial lesions are present, providing an evidence-based diagnostic tool. Nf-L and tau are the most abundant cytoskeleton proteins in the CNS and the peripheral nervous system (PNS). Elevated levels of these proteins are related to brain trauma, neurological disease, and repeated concussions. Quantification of these serum biomarkers among military personnel assists in determining injury effect in the absence of visual trauma [123].

5.2.2 Etiology and Epidemiology

The most common cause of TBI in the military is blunt impact to the head or active impact due to "whiplash." Blast injury accounts for ~60% of all military TBI and 80% of mTBI [124]. Walter Reed Army Institute of Research acknowledged that in 2020, TBI was increasing as well as the opportunities available for therapeutic interventions [125]. Researchers from the Institute's Neurotrauma Branch are exploring experimental animal studies and the effects of blast exposure. This has given them an understanding of the two phases of post-injury responses and the window of treatment. In 2019, the Research Transition Response team partnered with the U.S. Army Medical Material Development Activity to gather feedback at eight bases in Kuwait, Afghanistan, and Iraq on the effectiveness of two TBI-detection devices.

Since 2014, the DoD and the National Collegiate Athletic Association (NCAA) have partnered on this issue via the NCAA-DoD Concussion Assessment, Research, and Education (CARE) Consortium. With over 30 colleges and universities participating, including four military service academies, the Consortium is the largest study of its type to improve understanding of the effects of concussions and repetitive head impact exposures on brain health. CARE is the first concussion study to include both women and men across 24 sports. The mission is to gain a better understanding of the biological and physiological effects and the symptoms present for the course of recovery.

According to Dr. Paul Pasquina, Department Chair of Physical Medicine and Rehabilitation at the Uniformed Services University of the Health Sciences and Department Chief of Rehabilitation at Walter Reed National Military Medical Center, the next phase of the Consortium will include the Explosive Ordnance and Disposal School at Eglin Air Force Base, FL, the Defense Health Agency's National Intrepid Center of Excellence for TBI, and the Intrepid Spirit Centers, located at Fort Hood, TX, Fort Bragg, NC, and Joint Base Lewis-McChord, WA [126]. The partnership and collaboration of data open a gateway that will benefit more than student-athletes in the NCAA and provide benefits to military personnel.

Current nanoneuroprotection sensor research aligns with American football players in the realm of head injury, reporting real-time impact detection to understand the exposure of injury during a game or for the Warfighter in combat. Ph.D. student Jake Merrell and researchers at Brigham Young University have created a nickel nanocomposite "smart foam" called ExoNanoFoam to better monitor the force of impact on the brain through electrical signals [127]. The charge is calculated through electrodes in the foam measured by a microcomputer and transmitted in real-time wirelessly to a receiver such as a tablet for trainers. The spike in voltage is caused by impact. The advantages allow measurement of maximum acceleration, impact velocity, severity index, impact energy, and impact location, with 90% or better accuracy.

The development of nanocomposite foam (NCF) sensors demonstrates accuracy in measuring head injury criteria and Gadd severity index, as well as impact energy through triboelectric response. These sensors are designed with nickel NPs and nickel-coated carbon fiber in a liquid polyurethane foam matrix. The NCF sensors can be implemented into helmets, patches (skin), earplugs, skullcaps, mouthpieces, shoulder pads, and chinstraps, averaging $1,200 per helmet [127].

Michigan State University assistant professor Welyi Lu has been testing liquid nanofoam that, when under pressure, can conform to an object around it for greater protection [128]. The liner provides a thinner and less bulky alternative to traditional foam while still withstanding high-impact forces. The coated nanopores provide a hydrophobic silicone layer that prevents absorption by the material and becomes pressurized during impact. These future applications of nanotechnology go beyond athletic protection and will provide critical benefits when applied to the military setting.

TBI is increasing at an alarming rate. The CDC estimates the direct or indirect treatment cost to be greater than $76 billion in the United States. The need for effective diagnosis to decrease high error rates is made possible by BioDirection, Inc. [129]. They offer rapid POC/POI medical products to diagnose and manage TBI. The BioDirection Tbit System is a whole-blood POC test that is rapid, affordable, and generates a result in less than 2 minutes with just a single drop of blood. This system measures targeted brain biomarkers GFAP and S100B that are released following head trauma. S100B has been used clinically for the past 50–60 years worldwide. The Tbit System is designed with nanowires measuring 1/10,000 of a human hair for ultrasensitivity and specificity. This nanotechnology has the potential for upscale and affordable POC opportunities.

5.2.3 Trends

An ongoing DoD-funded project led by principal investigator Laila Abdullah, named "Identifying APOE-Related Lipid Biomarkers for Diagnosing Chronic Neurocognitive Deficits in TBI patients," focuses on the rehabilitation stage after a TBI, examining bioactive lipid metabolites (i.e., eicosanoids, isoprostanes, resolvins, lipoxins, ceramides, and sphingosine) for diagnosing mTBI and predicting cognitive decline [120]. As part of this project, nanoflow ultra-high-pressure liquid chromatography systems will be used for molecular measurements due to their extreme efficiency compared to standard systems. In addition, high-mass accuracy and high-resolution capabilities of the Q-Exactive hybrid quadrupole Orbitrap mass spectrometer will be used. The proposed work targets subjects collected in the Chronic Effects of Neurotrauma Consortium and military cohorts.

mTBI classifies 90% of all brain injuries in the Warfighter. For perspective, over 300,000 troops from OIF/OEF reported a form of TBI, of which 30% now suffer from post-traumatic stress disorder. This overlap is also common with the risk development of Alzheimer's disease (AD).

Blood biomarkers are characteristics that measure the difference between healthy and unhealthy individuals directly associated with managing the disease. Blood Aβ and tau levels are elevated in individuals with TBI of all severity levels. Evidence-based studies reported individuals with TBI and the apolipoprotein E (APOE) ε4 allele experience learning and memory impairment consistent with AD. The correlation between TBI and AD proves to be a medical research advantage and challenge as clinical trials are navigated. Blood biomarkers are the next step to detecting preclinical AD in Warfighters with TBI. According to Abdullah et al., "Omega-3 and omega-6 polyunsaturated fatty acid content within blood phospholipids (PLs) are altered in ε4 carriers with preclinical mild cognitive impairment (MCI)" [120]. This results in generating bioactive lipid metabolites that trigger inflammation and oxidate stress. Elevated sphingomyelin also influences the inflammation response.

Brain temperature is often used as a diagnostic indicator for neurological disorders. The temperature of the brain gauges neural activity, demonstrating activation and silencing [130]. Scientists, led by Swinburne Postdoctoral Research Fellow, Dr. Blanca del Rosal Rabes, have created a noninvasive, thermosensitive NP to measure subdegree brain temperature changes using near-infrared (NIR) light. Fluorescence nanothermometry in the second near-infrared window (NIR-II, 1,000–1,700 nm) allows for contactless measurements through the skull and skin. Current methods rely on implanting invasive probes (thermocouples and fiber optic sensors) involving direct contact with brain tissue, which requires a hole in the skull, creating the risk of organ damage. Magnetic resonance spectroscopy (MRS) is currently used for thermal mapping and accounts for small temperature variations (<1 °C). The limitation of MRS is its cost effectiveness, variations in tissue magnetic susceptibility, and limited spatial resolution.

The nanothermometry approach in neuroscience avoids damage to the brain and high spatial resolution with deep tissue penetration of NIR-II. In a study by del Rosal and colleagues, they discuss the preclinical benefits of contactless brain thermometry techniques provided by NIR transcranial temperature sensing [130]. Ag_2S (silver sulfide) nanodots (NDs) were used to exhibit high penetration depths with minimal light-induced heating close to room temperature. The linear intensity originates from "the direct relations between temperature and the probability of radiative de-excitement" [130]. Mice were intracerebrally injected with Ag_2S NDs and then analyzed through the contactless intracranial route for an in vivo fluorescence image. Brain temperature and electrical activity were monitored and showed suppression in brain activity, paired with a small temperature decrease. This correlation,

coupled with a contactless brain thermometry technique, delivers a valuable approach to enhancing diagnoses of brain injury and neurological trauma.

In laboratory studies, brain hypothermia was shown to be the most powerful neuroprotectant to limit destructive physiological cascades that result in cellular injury. Cerebral circulation regulates the thermal environment of the brain. After an injury such as TBI, the cerebral blood flow is reduced. This leads to secondary injury inhibiting recovery. According to Wang et al., "On average, 0.66 J is released every minute per gram of brain tissue, and if not properly regulated, it will accumulate and continue to increase local brain temperature" [131]. Following TBI, neuroinflammation and oxidative stress are triggered, leading to a secondary injury that causes cognitive and motor dysfunctions.

The role of ROS and oxidate stress are vital to cell survival, cell death, differentiation, and inflammation-related factor production. "Mitochondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) generate superoxide (O_2•–)" [132], a free radial involved in ROS. Due to its short life span, it causes the rapid reduction of hydrogen peroxide, a nonradical derivate of ROS. Superoxide production is the main antioxidant defense in cells exposed to oxygen. The balance of ROS generation and the counterbalance of excess ROS by cellular antioxidant factors cause a reduction-oxidation (redox) imbalance, leading to a disruption in homeostasis and resulting in damage to biomolecules, such as proteins, lipids, and nucleic acid. NP exposure in cells at low concentration shows powerful antioxidant abilities to overcome oxidative stress and redox balance, unlike high exposure to NP concentration that overwhelms the antioxidant system contributing to cytotoxicity and inflammation. This, in turn, is a beneficial component in treating cancerous tumors. Finding the balance of NP through nanoceria (cerium oxide NPs) exposure leads to suppression of adipogenic differentiation, inhibiting the ROS generation. Nanoceria redox properties mimic superoxide acting as a good therapeutic alternative to ROS mediated diseases.

Preclinical studies later followed by clinical trials discuss the transplantation of human mesenchymal stem cells (hMSCs) for regenerating lost tissues for patients with TBI. Limitations were the time of transplantation, number, and quality of transplanted cells and proinflammatory of injured parenchyma [133]. In the study by Mayilsamy and colleagues, they developed a nanocell therapy using dendrimer composite with plasmids (dendriplexes) targeting CCL20 and its sole receptor CCR6 to reduce inflammation followed by hMSC transplantation [134]. Dendrimers are hyperbranched polymers used for molecule encapsulation surrounding a central core for high-drug load capacity. Polyamidoamine (PAMAM) NP dendrimers can circulate into the CNS and cross the BBB for targeting inflammation. PAMAM G4 dendrimer was coupled with a vector-based, short hairpin RNA (shRNA) encoding plasmid, resulting in a successful dendriplex formation, with intended gene silence. The gene silencing by shRNA offered a target-specific protein expression toward CCL20 and CCR6. The fluorescence cy7 was attached for imaging purposes, which found PAMAM highly distributed in the brain and liver after being administrated intranasally (IN) and intravenously (IV). For this study, the fluorescence was still observed 72 hours after administration in both locations.

Previous studies showed NP-mediated delivery using PAMAM dendrimers to be more effective IN to deliver to the brain than IV and considered nontoxic at low doses. When the mice in the study were compared between CCL20 and shCCL20 on the seventh day, there was a significant decrease in inflammation in the mice treated with the shCCL20 dendriplex. The same was observed with the shCCR6-treated animals. Although the regeneration potentials of MSCs after TBI

is promising, it is important to note that these cells are immune-privileged, immunotolerant, and inhibit T-cell alloreactivity. rTBI causes the production of CCL20, a process toward secondary neurodegeneration and neuroinflammation. It is reduced by hMSC therapy when coupled with pioglitazone (PPARγ agonist), which improves the efficacy of the transplant. In addition, to the gene silencing of CCL20 and CCR6 with the hMSC transplantation, there was an increase in brain-derived neurotrophic factor (BDNF). BDNF plays a role in cell growth, indicating that at the early stage after injury, the brain is already preparing for the repair process [134].

In 2019, TBI was estimated to affect 12%–16.7% of males and 8.5% of females, mostly occurring in 15–30-year-olds [135]. Cognitive disorders that follow TBI can significantly impair quality of life, demanding innovative technologies. Robotics and virtual reality (VR) allow a smaller workforce, with multisensory stimulation over long periods. Lokomat is a robotic device used in the rehabilitation field to introduce a modern treatment alternative. The VR screen is electronically controlled with an avatar to mimic immediate visual movement feedback of the body. Robotic rehabilitation originally focused on limb functions. Lokomat Pro and Lokomat Nanos can evaluate cognitive and emotional behaviors. Current operational functions for Lokomat use a motorized gait insole with computer-controlled linear actuators on each hip and knee joint, mechatronic body weight support (BWS), and a treadmill. These patterns are adjusted to the patients' needs for 30-minute sessions, evaluating the physiological rigidity and sometric force exerted. BWS supports 20%–70% of the patient's weight. Activities through VR include patients collecting and/or avoiding objects like video games strategies. Studies show cognitive and motor rehabilitation programs are extremely beneficial for recovery, while improving patients' mood and quality of life. The combination of Lokomat Nano and VR stimulates brain repair through improved global cognitive functioning, attention, and executive process. These functions significantly enhance the patient's autonomy and rehabilitation results and prove to be essential for clinical recovery in TBI patients.

5.2.4 Future Opportunities and Challenges

There is a lack of an objective POI or POC test. TBI testing today is a symptomatic diagnosis, which has a high error rate and is not at all definitive [129]. As of 2020, there was only one TBI biomarker-based diagnostic on the market. It uses enzyme-linked immunosorbent assays to measure the breakdown of GFAP and UCH-L1 [136]. Another limitation with blood biomarkers is they must be collected in the early phase following the trauma. Saliva and plasma represent most of the specimens, but new research has been focused on cerebrospinal fluid for more rapid and specificity of imbalances in the neural pathway [137]. Although NPs have been proven to be a rapid and effective biomarker, further studies are needed to determine their toxicity and bioaccumulation in clinical settings [138]. Moreover, further studies are needed to understand the mechanisms of NP-induced ROS. NPs have proven to be a rapid and effective biomarker for brain disease and disorders. To ensure the effectiveness and efficiency of these particles, the properties of NPs modify the ROS effect. NP-induced ROS production displays various functions and medical applications for stem cell biology [132].

The major target for therapeutic NP delivery is to provide neuroprotection and reduce cell disruption by preventing the spread of secondary injury beyond the primary contusion area [122]. TBI has been identified to be among the risk factors for neurodegenerative diseases, including AD, Parkinson's disease (PD), and others. Research for Aβ plaques commonly associated with AD, α-synuclein to PD, and TDP-43 in amyotrophic lateral sclerosis and frontotemporal dementia all show a relationship that suggests TBI patients with long-term adverse neurological outcomes are at an increased risk of dementia [124]. There

is also research on autism spectrum disorder that uses nanohesperetin to restore behavioral defects and suppress inflammation and oxidative stress, which could be explored for a role in secondary injury. Nanodopamine is associated with PD, as it improves motor defects with low toxicity [139]. The therapeutic roles for other neurodegenerative diseases show promising results for long-term care in TBI and should be integrated to facilitate patient outcomes in clinical recovery.

5.3 NEUROPROTECTION AND MONITORING

5.3.1 Overview

Biosensor technology has prompted researchers to develop biosensors for detecting biomarkers. The spotlight has focused on finding a cost-effective application, with high sensitivity and specificity. Other implementations of nanotechnology include nanowires, carbon nanotubes, devices, and sensors to protect, repair, or regenerate brain function and circuity [140]. Primary injury in neurotrauma is a result of high force on the brain. Secondary injury gradually occurs as an outcome of catastrophic cellular and molecular events. Current treatment does not include the prevention of a pathological cascade that causes a secondary injury [141].

5.3.2 Current Research

The Office of Naval Research, through the Defense Research University Instrumentation Program, has supported funding for Rice University to invent Warfighter protective technology. Through the $1.3 million grant, they have created the world's first printable military "smart helmet" using industrial-grade 3D printers. Led by principal investigator Paul Cherukuri, Executive Director of Rice's Institute of Biosciences and Bioengineering, "the Smart Helmet program aims to modernize standard-issue military helmets by 3D-printing a nanomaterial-enhanced exoskeleton with embedded sensors to actively protect the brain against kinetic or directed-energy effects" [142]. The Carbon Inc.'s L1 printer builds a strong but light military-grade helmet that combines nanomaterials, artificial intelligence, haptic feedback, image processing, and energy storage. This program incorporates technology from the FlatCam project led by Ashok Veeraraghavan and colleagues, which integrates image processing to eliminate bulky lenses. The Smart Helmet also embodies Cherukuri Teslaphoresis's design, a "tractor beam" for nanotechnology that creates physical and electromagnetic shields inside the helmets. According to Cherukuri [142], "We're simply developing that technology into a device that gives the men and women protecting our country a real chance at coming home safe and sound. This is for them."

The NanoDx System partners with IBM research for the use of its complementary metal-oxide semiconducive compatible nanosensors to provide real-time assessment of injury or infection with higher sensitivity and specificity than other POC tests. In a POC test, "The unique size and the electrical property of the nanosensors allow detection of biomarker and infections never before possible" [143]. The NanoDx design is built with highly sensitive nanowires that measure electrical resistance and preforms with antibodies that bind with the target biomarkers with a 98% accuracy rate. Although the NanoDx System has not been evaluated by the FDA or other regulatory agencies, the future application aims to help 634 million people who may experience TBI, strokes, sepsis, and COVID-19. Specifically, TBI diagnostics use the presence of biomarkers proteins (S100B and GFAP) in the blood and can measure the severity on a scale from mild to severe.

5.3.3 Trends

Nucleic acid-based therapeutics, such as small interfering RNA (siRNA), target specific pathological pathways to silence genes in efforts to mitigate injury progression. A biological barrier for the delivery of siRNA into the brain is the BBB. The vulnerability of the BBB in TBI is diverse and will vary on the severity of the primary injury. The BBB has a unique characteristic and can self-heal within

a few hours to days after injury, limiting the window for interventions. This is where nanotechnology plays a role in maximizing active penetration across the BBB. NPs formulated "using poly (lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer that exists in several Food and Drug Administration approved products, were more efficient when administered during early or late injury period" [141].

In the study by Li et al. [141], the accumulation of NPs in the BBB depended on the type of surface coating and the coating density (Figure 5-1). This also affected the efficiency in the uptake of siRNA-loaded NPs. The surface coatings that demonstrated high encapsulation efficiency (55%–65%) were polysorbate 80 (PS 80), poloxamer 188 (Pluronic F-68), DSPE-PEG-glutathione (GSH), and DSPE-PEG-transferrin (Tf). Subsequently, cellular uptake is the challenge of the release of siRNA from the encapsulated endosome. This study used LysoTracker green—a fluorescence probe—to label endolysosomes. After two hours, the separation of siRNA (red) and endolysosomes (green) occurred. Additional research compared the penetration of Dy677-labeled siRNA-loaded PS 80 (H)-NPs and free siRNA in an induced TBI model for early and late injury. There was a "strong fluorescence signal

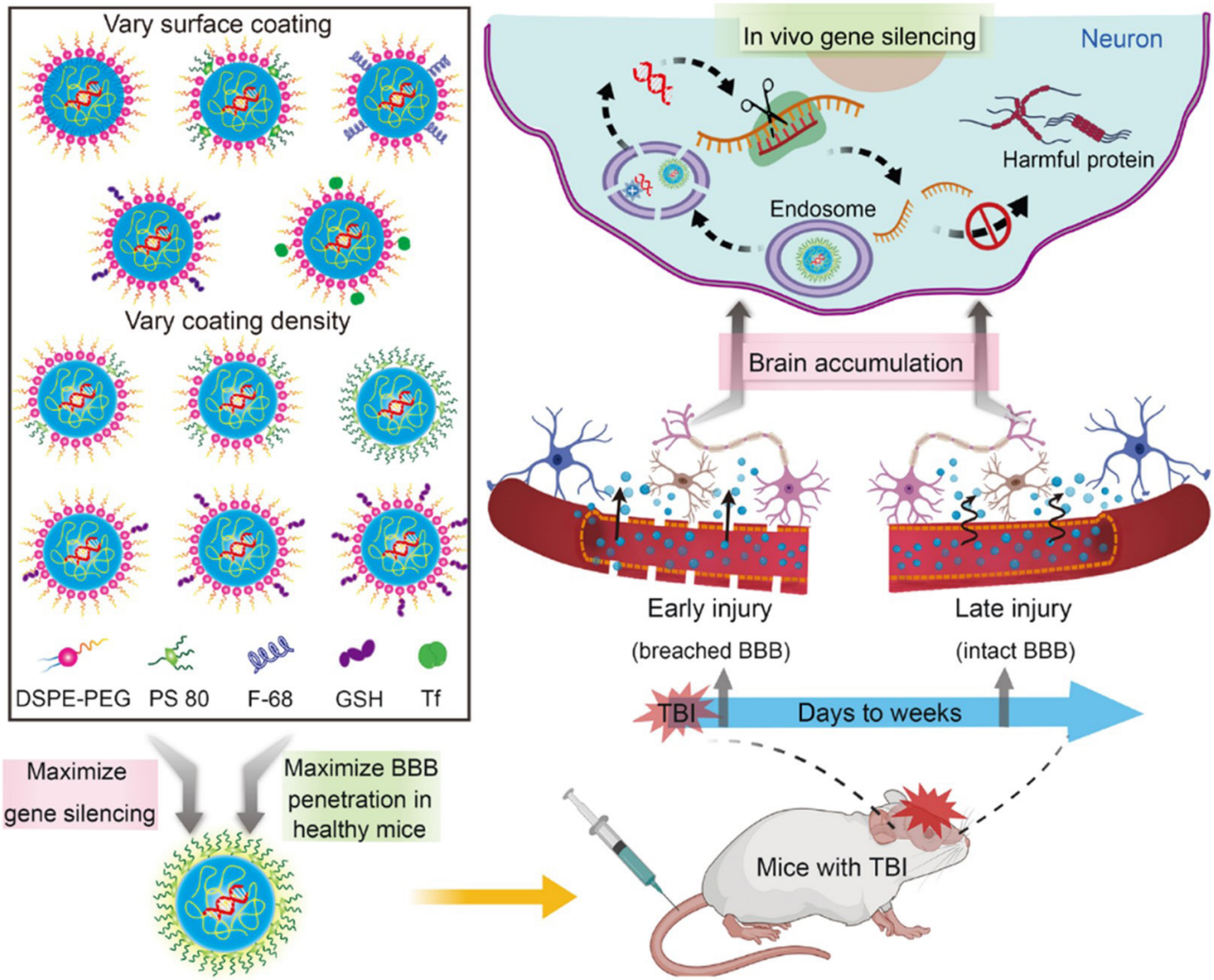

Figure 5-1. NPs With Different Surface Coatings Were Prepared to Achieve BBB Pathophysiology–Independent Delivery of siRNA in TBI *(Source: Li et al. [141]).*

in the brain of mice injected with siRNA-loaded PS 80 (h)-NP, with a fivefold and threefold higher fluorescence intensity when compared with free siRNA and siRNA-loaded PEG-NP groups" [141].

To evaluate late injury, once the BBB self-repairs, both free siRNA and siRNA-loaded NP were administered intravenously two weeks after injury. Again, the results showed siRNA-loaded PS 80 (H)-NPs with high fluorescence signal as an effective delivery into the BBB. These results hold great potential for regulating pathological targets involving early or late injury, which could last weeks to months. A safety profile and systemic toxicity of PS 80 (H)-NP was conducted in this study, stating biochemical parameters confirmed no pathological changes in major organs including lung, heart, liver, spleen, and kidney [141].

The endocannabinoid system (ECS) is a biological system consisting of endogenous lipid-based retrograde neurotransmitters that bind to cannabinoid receptors (CBRs) and enzymes expressed through the CNS [140]. The recognition of CBRs originated from understanding the psychoactive effects of tetrahydrocannabinol (THC), the component of cannabis. ECS exists and is active in the human body, regardless of cannabis use [144]. Howlett et al. established that "cannabinoids activated a G protein-coupled receptor (GPCR) that inhibited adenylyl cyclase" [145].

The ECS has several roles—regulating cognitive and physiological processes, including pregnancy and fertility, pain sensation, appetite, mood, and memory, and mediating the pharmacological effects of cannabis and its derivatives [140]. The system is also involved in several disorders and injuries, including spinal cord injury, attention-deficit/hyperactivity disorder, and sleep deprivation-related issues. There are two types of ECS receptors—CB1, which is mostly found in the CNS, and SB2, which is found in the PNS (immune cells). An example of endocannabinoids in action is they might bind to CB1 receptors in a spinal nerve to alleviate pain, and the CB2 receptors might bind to immune cells to signal inflammation [144]. There are three types of cannabinoid compounds—anandamides or arachidonoyl-ethanolamide (AEA) and 2-arachidonoylglyerol (2-AG), which are naturally produced by the body; phytocannabinoids (9-THC); and cannabinols, which are naturally found in the cannabis plant and man-made synthetic cannabinoids.

Mobed et al. [140] focused on 2-AG and the primary endogenous ligand (nuclear receptor) for the CB2 receptor. The urge for more cost-effective and sensitive advantages to the medical industry has led researchers to the field of nanotechnology. Nanomaterials such as gold nanoflowers (Au NFs) have a high surface-to-volume ratio, conductivity, and optoelectronic features considered desirable biosensors for detecting biomarkers. Applications for the Au NFs in biosensing depend on the pH and work best with lower concentrations of citrate for the structure of the gold wires and morphology of the Au NPs. The primary bonds in bioconjugation of Au NP are covalent and noncovalent. As a result, the electrostatic interaction changes the property of the Au NP's size and neuroanatomy. Field emission scanning electron microscopy is commonly used to identify the applied nanocomposite in biosensors. In this study, Au NP at pH = 6.14 forms a flower-like structure and produces an aggregative structure at pH = 4.19. As reported, Au NP was used to reach long-term stability and reliability. The cost-effective and fast-acting sensing system developed could replace current technology to "open up new horizons in the design of immune platforms for the monitoring of 2-AG in human biofluid and rapid screening of immunomodulation and neuroprotection" [140].

Curcumin (diferuloylmethane), commonly known as a spice or dietary supplement and a component in turmeric, is a natural antioxidant. In both preclinical and clinical studies, curcumin has expressed neuroprotective behavior against various neurodegenerative diseases for impaired learning and memory parameters. Challenges

that have been presented in clinical studies are "low solubility, physicochemical instability, poor bioavailability, rapid metabolism, and poor pharmacokinetics" [146]. Throughout recent studies, nanocarriers increase efficacy of curcumin for improving therapeutic activity. NanoCurcTM, polymeric NPs encapsulated in curcumin, provided neuron protection in vitro as well as in vivo studies. According to Naqvi et al., "In vivo, intraperitoneal (IP) injection of NanoCurcTM at a dose of 25 mg/kg twice daily in athymic mice demonstrated significant curcumin levels in the brain and resulted in decreased levels of H_2O_2 and caspases activities in the brain, along with increased glutathione concentrations" [146]. Similar treatment showed protection in human SK-N-SH cells from hydrogen peroxide intervening in ROS. The attraction to curcumin research stems from its benefits in neurodegenerative disorders such as stroke, tumors, multiple sclerosis, epilepsy, Huntington's Disease, PD, and AD. The same nanotechnology can be applied to patients with TBI for the delivery of therapeutic drugs for long-term care [146].

5.3.4 Safety and Regulatory Challenges for Nanoprotection and Monitoring

Nanotechnology-based polymers such as poly (alkyl cyanoacrylate), polyesters such as poly (lactide), PLGA, liposomes, solid-liquid NPS, micelles, nanoemulsion (oil in water), and dendrimer are all effective drug carriers. The drug delivery mechanisms of nanocarriers have selective access to the brain either by passive, gradient-dependent (passive targeting) or active, energy-dependent (active targeting) pathways. The presence of tight junctions in the brain requires the specialty size of NP for transmission past the BBB. The major developments of nanomedicine continue to be safety and high efficacy, drug targeting to specific sites to reduce off-target toxicity, and improved pharmacokinetic behavior by sustained drug release with a wide safety margin. The nanocarriers should be biodegradable, nontoxic, biocompatible, cost-effective, and site-specific. Clinical studies lack information about "fate in terms of toxicity, aggregation, and rapid clearance due to nano size" [146]. Toxicological studies of brain targeting nanoformulations should be further investigated and evaluated. The cellular level of neuroscience and neuroengineering shows promising future applications related to nanoscale bioengineering.

5.4 NANOTECHNOLOGY FOR PAIN MANAGEMENT

5.4.1 Overview

To better address pain management and develop new therapies, it is imperative to understand the physiology of pain. The complex nature of pain makes it laborious to treat, as it is measured subjectively between patients. According to the International Association for the Study of Pain (IASP), pain is defined as an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage [147]. Involving nanotherapeutics pain, physiology and psychological factors must be considered when providing pharmaceutical treatments [148]. Current pain management is treated with novel methods that lack specificity. Interventions that follow this current style begin with nonopioid analgesics like acetaminophen, nonsteroidal anti-inflammatory drugs, and adjuvant medications. Long-term use of these medications can cause negative side effects, such as hepatotoxicity, kidney damage, myocardial infarction, and a heavy drug load. The next level of pain management is opioids; the most used are morphine, fentanyl, hydrocodone, and oxycodone. Although opioids have been considered the most effective pain medication, they are coupled with severe side effects and a high abuse rate.

Scientists are applying nanotechnology in the medical field to improve current pain management strategies, create safer drugs, an alternative to opioid treatment, and assist in lowering the addictive aspects of opioids. The design of nanomedicines enhances the efficiency and safety of the drug through their size, shape,

surface charge, and high drug cargo for prolonged circulation. The biocompatible nanocarriers target pain-signaling receptors, allowing them to localize at the source of pain and reduce drug load. The nature of the nanoparticulate drug delivery systems (NDDSs) allows for multiple administration routes, including oral and nasal, intravenous, intrathecal, and transdermal, pushing nanotechnology as a great candidate [148]. The DoD is transitioning toward analgesic treatment in nonopioid forms. The research reflects the benefits of pain management at a nanoscale, focusing on biological-based treatments and opioid alternatives.

5.4.2 Trends

The shape of NPs enables them to be the desired application for pain management through their effective drug delivery and reduced drug load. The geometry of an NP allows it to be a beneficial host in an application for pain management. Nanocarriers are used as an effective drug delivery system for the treatment of pain management through comfort in neuropathy, systemic medication, and inflammation-related pain by targeting molecules. According to Bhansali et al., "Nanomedicine aims to apply nanotechnology to enhancing the efficacy and safety of drugs, for example, by encapsulating naked drugs in biocompatible nanocarriers such as NPs, liposomes, micelles, and dendrimers" [148]. Trends in nanomedicine circles around drug delivery are characterized by the ability to target specific tissues, the central nervous system, and prolonged drug circulation. Analgesic NDDSs (Figure 5-2) can include intranasal, intraoral, intrathecal, and transdermal administration. Local anesthetic loaded NDDSs via injection can block pathways related to perioperative pain and proves useful for extended treatment. This technology uses small molecules, allowing for reduced dosage and localizing the efficacy that targets pain signaling receptors. An active area of research is targeting opioid receptors to create safer and less addictive opioids while moving toward a more effective delivery. In addition, new research is being explored with theragnostic NPs to detect the source of pain. This emerging development can serve as an analgesic alternative with a reduced risk of opioid addiction.

The conventional pain management treatment plans typically involve the intermittent administration of unencapsulated drugs, which can cause an undulation in plasma drug concentrations. Fluctuations in plasma drug concentrations are affected by the bioavailability, dosage, route of administration, and absorption of a drug. This irregular pattern may affect clinical response and tolerability [149]. An approach with nanomedicine is the use of liposomes and polymerics to "encapsulate opioids for extended release (ER) and reduce systemic toxicity" [148]. Furthermore, the FDA has approved the commercialization of two ER morphine NDDSs—Depodur and Avinza. Commercialized, extended-release profiles have been broadly studied and considered safe carriers at therapeutic concentrations and stabilized plasma drug levels. Another approach to countering opioid abuse and heightened pain medicine tolerance is leu-enkephalin (LENK), which is an endogenous opioid peptide neurotransmitter joined with lipid squalene to target proinflammatory mediators. The nanoformulated, linked LENK-squalene has a threshold of a higher drug payload than enkephalin (ENK)-loaded liposomes or PLGA NPs. A barrier that surpasses with LENK-squalene is the ability to deliver microparticulate intranasally as clustered NPs specifically to the brain. Animal studies show positive results with intravenous administration of LENK-squalene NPs for a "greater antihyperalgesic effect than morphine, without causing tolerance" [148].

Mesoporous silica NPs (MSNs) are versatile drug carriers that enable multistage drug delivery. Referred to as stimuli response, this advancement demonstrates different behaviors during systemic circulation. It is classified into two categories—physical stimuli (magnetic, ultrasound, and external

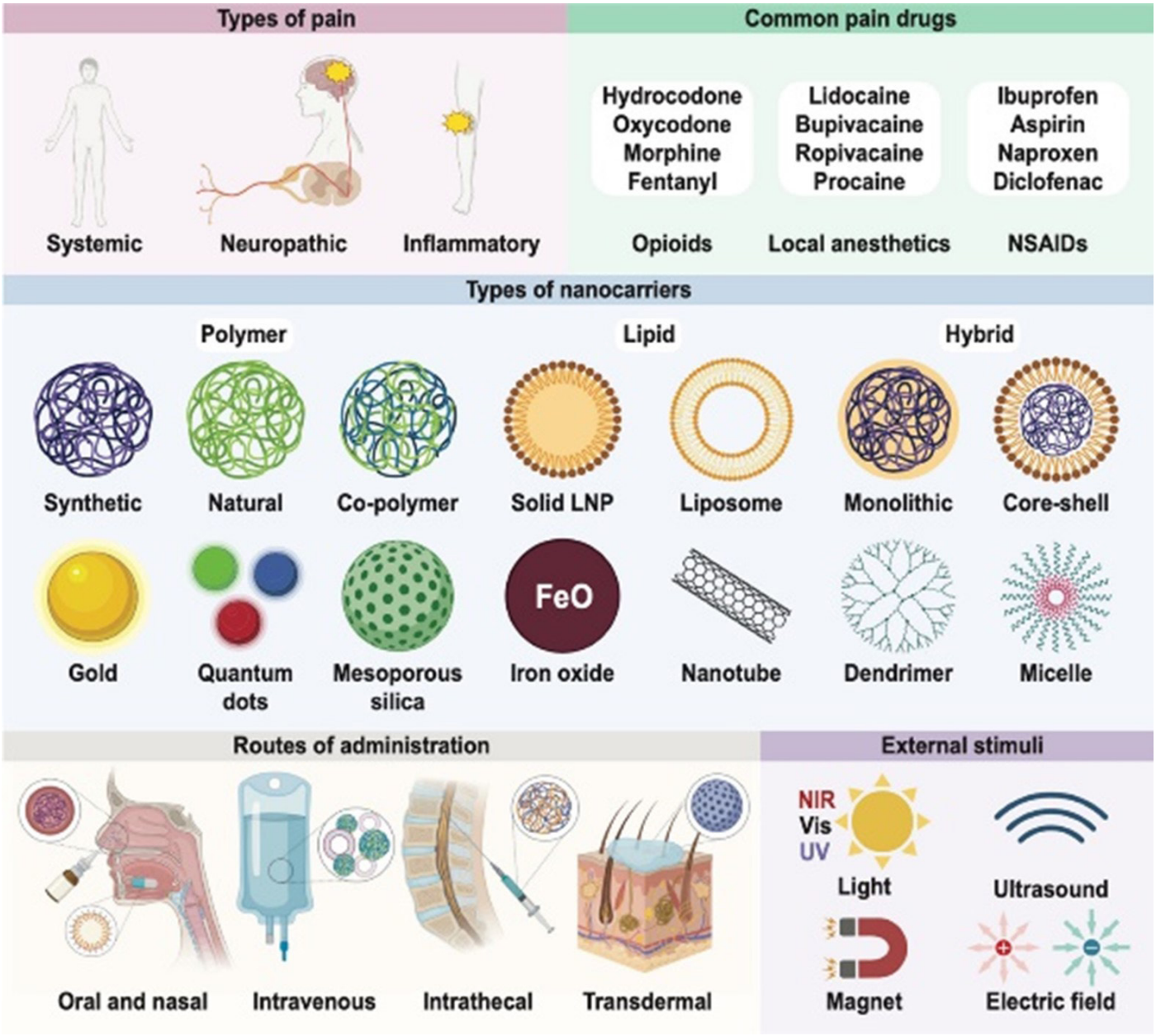

Figure 5-2. NDDSs *(Source: Bhansali et al. [148]).*

light) and intracellular biological stimuli (pH, hypoxia, and reactions with enzymes) [150]. MSNs' internal cylindrical pores and exterior particle surface proves to be a promising vessel. PEGylated liposome coating (lipoMSN) is stable and long circulating to allow long-term inflammatory pain relief. To this point, Bhansali et. al [148] noted that:

> *"pH-responsive MSNs functionalized with a PEGylated liposome coating (lipoMSN) and loaded with a δ-opioid receptor agonist DADLE ([D-Ala2, D-Leu5]-Enkephalin) can target endosomal δ-opioid receptors and provide sustained inflammatory pain relief. The pH-responsiveness of the lipoMSN allows for preferential delivery to the acidified endosome while the DADLE-functionalized liposomal coating helps to cloak the MSN core and selectively target δ-opioid receptor-expressing neurons."*

Previous research shows one intrathecal injection of the lipoMSN can provide analgesia in a mouse model of inflammatory nociception for six hours. The results suggest a correlation between δ-opioid receptors and endosomal signaling, an opportunity for NDDSs because of the natural transporting of NPs to endosomal signaling [148].

Evidence-based studies show inflammatory responses related to trauma are initiated through the activation of microglia and macrophages, revealing phagocytic activity. This leans on the same fundamental finding that the shape of NPs has a major role in their effectiveness for drug delivery and reduced drug load. Chakravarthy et al. [151] stated the long-term effects of the phagocytosis (an actin-based process) relationship to actin remodeling within the cytoskeleton of macrophages, the excretion from the body related to the toxicity, and the clinical advantage of targeting is an opportunity in research. Nanotechnology can lower the expression of proinflammatory peptides while diminishing the side effects of neurodegeneration and chronic neuropathic pain. Research has been developed in advanced nanomedical membrane technology using nanofluidic channels as a drug delivery system. The application of an electric field (at ~1.5 V and 100 nW) between two platinum electrodes and seated in a nanoporous membrane is responsible for the molecular control [151]. Clinical trials are still in the beginning stages and expected to find a strong association for next-generation pain therapies to alleviate chronic pain.

5.4.3 New Targets in Nanopharmaceuticals

Nanopharmaceuticals have been expanding in active research, with the drive to effectively manage pain while decreasing opioid administration. Fentanyl citrate has proved to be a promising direction when coupled with a NP-based delivery system to enhance the transmucosal transport/ bioavailability of fentanyl. This formalization of a drug that possesses a high surface area-to-volume ratio improves the overall efficacy via enhanced fast-acting absorption and pharmacokinetics as it bypasses the gastrointestinal tract and first-pass liver metabolism. Chakravarthy and colleagues [151] referenced a clinical trial that evaluated the success of oral transmucosal fentanyl citrate when compared to a placebo-controlled study using immediate-release morphine sulfate (MSIR). The randomized double-blind crossover study ($n = 93$) was statistically, significantly favoring MSIR in terms of pain intensity difference ($P < 0.008$) and pain relief ($P < 0.009$) at each time point (15-, 30-, 45-, and 60-minutes post-dose ingestion). Transmucosal transport can be administered intranasally, orally, or rectally. This alternative provides a needle-free delivery favorable to combat care in military medicine.

Ketamine can be coadministrated with a strong opioid with an analgesic, adjuvant drug for pain management. Han et al. [152] focused on the development of biocompatible and biodegradable ketamine-loaded, PEG-block-PLGA NPs with an abnormally high drug cargo for sustained release. PLGA is an FDA-approved polymer with biodegradability and biocompatibility. It has been shown to be effective when used for drug loading NPs with controlled release. When coupled, the PLGA forms "core-shell structured NPs to encapsulate a variety of therapeutic compounds" [152]. PEG-PLGA NP has a hydrophilic shell, allowing it to carry insoluble drugs. The stability of the ketamine-loaded NP showed an unchanged size for seven days, confirming their findings that PLGA, in combination with PEG, can improve drug release profiles. The study showed that during in vitro release, the ketamine was prolonged over a desirable three-week period at a steady rate. This length depended on pH-sensitive polymer, showing favorable acidic conditions. In vivo, the pharmacokinetic parameters of ketamine and the ketamine-loaded NP half-life of release were compared. The ketamine-loaded NP withstood a longer half-life of release than ketamine alone at five days. The incorporation of NP increased half-life by 20%, the shape, particle size, and type of polymers affected the degradability and

drug release kinetics. The characteristics of the encapsulated NPs enabled the controlled slow release. Extended release with a high drug cargo can demonstrate lasting pain management for military use. Overall, this nanotechnology can be applied to the application in the DoD through combat care.

The DoD is funding an ongoing project for long acting, nonopioid analgesic (LANA) nanomedicine to specifically target cells involved in neuroimmune response to peripheral nerve injury. LANA nanomedicine has the potential to extend pain relief for >72 hours upon single, low-dose administration. The target drug delivery technology of NPs can accomplish an increase of overall effectiveness of the drug by up to 100-fold, promote healing, and drastically minimize drug side effects. Throughout this study, the aim is to evaluate safety and establish efficacy to produce a scale-up nanomedicine drug product (LANA) for FDA approval in pain management [153].

5.4.4 Safety and Regulatory Challenges for Nanotechnology

Nanomedicine in the application for temporary or long-term reduction or cessation of pain is still in its nascent stages. Various evidence-based studies show good measures and powerful results to significantly diminishing pain and nanotechnology as a superior drug delivery system. Clinical trials indicate the strong prospective future for nanocarriers such as liposomes and polymeric NPs in efficacy, extended-release, drug safety, and patient adherence. Protocols must be applied to ensure efficacy and optimal safety (physiological and environmental). Chakravarthy et al. suggested novel nanomaterials with new properties (e.g., morphological, electronic, physicochemical, biological, and functional kinetics) should be compared to their macroscale/bulk counterparts to establish and standardize desirable protocols and all-inclusive assessments [151].

When using nanotechnology, the risk for a negative immune response is possible, as NP could be identified as foreign and therefore the treatment be rejected. The true response is unknown until further clinical testing can be performed and evaluated. In the interim, case-by-case evaluation systems to distinguish hazardous nanoscale effects, such as a database to classify the unique features of specific NPs and other nanometric entities, could be used to establish and standardize protocols [151]. Another challenge faced with nanomedicine is the limited research conducted on NP passing the BBB. In succeeding studies, "positively charged nanocarriers were used for delivery of opiate-related drugs and peptides into the brain" [154]. The amount that could transfer into the BBB was less than 1%; however, during the nascent stage of nanomedicine, there is potential for more research [154].

SECTION 06 ADVANCED MEDICAL MONITORING AND DIAGNOSTICS

6.1 OVERVIEW

Monitoring wounded personnel is one of the most important functions in CCC. As Bird and Ravindra suggest, "Functionalized multiscale biomedical materials, sensors and analytical devices that can better interface and integrate with the Warfighter will enhance the safety and well-being of service members giving them a distinct advantage over adversaries on the battlefield" [155]. DoD entities from the U.S. Army Combat Capabilities Development Command's Chemical Biological Center to the Defense Threat Reduction Agency are seeking to use bioelectronics to provide valuable information for acute care and transforming military medicine on the battlefield [155]. There should be a continuous feedback loop between physiological changes in the injured Warfighter and actions taken by medical personnel from the time of initial injury through the transfer to a military treatment facility in the continental United States. Although such continuity of care is currently imperfect, advances in nanotechnology are making this more of a reality.

In terms of advanced medical monitoring and diagnostics, the DoD is currently interested in improving en route care and medevac operations. For example, the mission of the Advanced Capabilities for Emergency Medical Monitoring program "is to conduct basic and applied research that leads to the identification and integration of physiological measures that reflect the complexity of compensatory responses by the body during the early dynamic phase(s) of hemorrhage" with the ultimate goal of "applying medical monitoring capabilities of combat medical personnel for triage, diagnosis and decision-making to improve the management of combat casualties" [156]. Activities leading to these advances include creating an ecosystem of biosensing (in real-time or near real-time) coupled with the ability to administer therapeutics in-kind and actively observe and respond accordingly throughout the process. The concept of biosensing in CCC is extending beyond just monitoring physiological change after injury to also include monitoring prediagnostic conditions (heat stress, altitude sickness, infection/chemical, biological, radiological, and nuclear [CBRN] threats), status of wounds and injury sites, and psychological states (Figure 6-1).

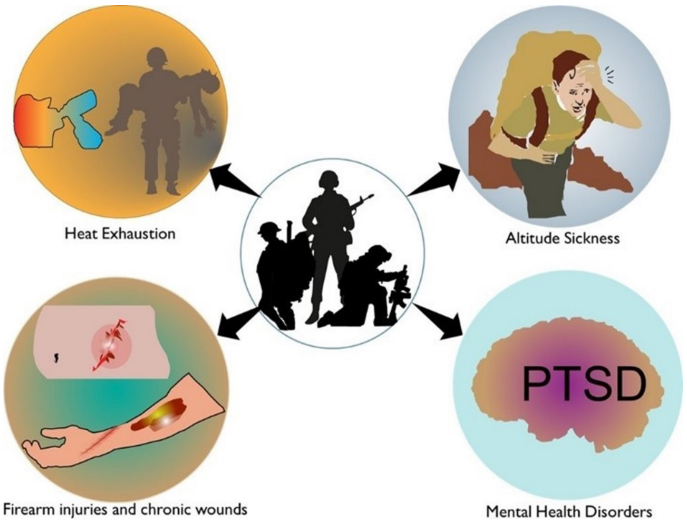

Figure 6-1. Biosensing Needs for Continuous Monitoring of Soldier Wellness in Harsh and Hostile Environments *(Source: Bhide et al. [157]).*

Many of these new realities are becoming possible through the design and use of nanosensors, which can "identify specific cells at the molecular level to deliver medicine and monitor the development of particular places in the body by measuring physical characteristics such as volume, concentration, movement and speed, gravitational, electric, magnetic forces, pressure, temperature, etc." [158]. Nanosensors are sensing devices made from or with engineered nanomaterials and can be manufactured with the following:

- Nanoscale wires.
- Carbon-based nanomaterials (carbon nanotubes [CNTs] and graphene [GR]).
- Thin films.
- Metal and metal oxide NPs.
- Nanoscale polymers.
- Nanobiomaterials.

Two of the most important and currently used and researched materials for nanosensors are carbon-based nanomaterials and metal NPs. According to Javaid et al. [158], carbon-based nanomaterials offer improved conductivity, stability, lower costs, and ease of surface functionalization compared with traditional sensor materials. Common carbon nanomaterials used in sensing include CNTs and GR, which have been evaluated for use in chemiresistive sensing. Some recent applications of carbon-based GR nanomaterials for biosensors are found in Table 6-1.

Metal NPs are another commonly used type of nanomaterial of biosensing. These types of sensors can often be more sensitive and selective due to the ability to use custom signal amplifications [158]. Commonly explored metals for this purpose include cobalt, Cu, Au, palladium (Pd), platinum (Pt), Ag, and others.

Table 6-1. Carbon-Based Nanomaterial Applications Used in Nanobiosensing *(Source: Bardhan et al. [159])*

Carbon-Based Nanomaterial	Detection Method (Signal Transduction)	Functional Modifications	Analyte of Interest (Applications)
Graphene/Graphene Oxide (GO)/Reduced Graphene Oxide (rGO)	Electrochemical (Reid Effect Transistor)	N-doping, surface attachment of anti-IgGs, Au NPs on graphene surface	Detection of VEGF and other cancer biomarkers, in situ sensing of biomolecules, DNA detection, DNA hybridization, in vivo imaging in living cells and in animals, protein detection, sensing of pathogens, detection of enzymes and small molecules such as H_20_2, NAO^+, dopamine, glucose, etc.
Graphene/Graphene Quantum Dots (GQDs)	Optical (fluorescence, photoluminescence, luminescence, FRET, surface plasmon resonance [SPR], surface-enhanced Raman spectroscopy [SERS], etc.)	ssDNA, gold on GO, aptamer with graphene composite	Detection of Hepatitis C virus helicase inhibitors, inhibitors of the SARS CoV coronavirus, detection of circulating tumor cells (CTCs), human IgG, proteins, toxins such as cholera, thrombin detection.
Carbon Nanotubes	Electrochemical (amperometric, potentiometric, voltametric, piezoelectric)	Enzyme coupling on working electrode, DNA wrapping	DNA detection (such as TP53 mutation), detection of biomarkers, volatile organic compounds (VOCs) in breath for cancer detection, early detection of PSA in serum for prostate cancer, glucose biosensor for diabetes monitoring, cholesterol biosensor, detecting cellular nitric oxide, H_20_2 production in the cancer microenvironment, etc.
Carbon Nanotubes	Immunosensing	Antibodies/antibody fragments, peptides such as RGDS	Detection of HIV virus, hepatitis, test for drugs and undesirable toxic compounds in the environment, detection of cancers such as head-and-neck cancer, breast cancer, etc.
Carbon Nanotubes	Optical (fluorescence, phosphorescence, absorbance, reflectance, surface plasmon resonance, quenching, life time, photoacoustic, etc.)	Peptide modification, stabilization with biomolecules such as ssDNA, M13 phage, or oligonucleotides	Studying spatio-temporal process and biomolecules in living organisms and in cells, detection of ATP in living cells, in vivo deep tissue imaging, early detection of cancers by fluorescence quenching or enhancement upon binding to up- or down-regulated cell surface markers, etc.

Based on a review of developments in the field, nanomaterials represent three key categories of sensor R&D—wearables, implantable sensors, and stretchable sensors/flexible electronics.

6.2 WEARABLES

Wearables have been an extremely popular class of devices in recent years, especially for uses in healthcare. Devices with specific health-related functions are designated as wearable health devices (WHDs). Global revenue for WHDs doubled from 2010 to 2018 ($6–$12 million) [160]. Their development has largely been from their versatility and multifunction use in a multitude of items, including watches, glasses, rings, and ear buds. WHDs can currently be used to monitor most physiological activities, including heart rate, cardiac rhythm, blood oxygen content, sleep patterns, glucose levels, and even mood, among other physiological markers [155].

The emergence of wearable technologies is changing the DoD's approach in providing diagnostic and therapeutic care [155]. In March 2022, USAMRDC hosted an inaugural virtual meeting on wearable technologies with researchers from the Israeli Defense Forces Medical Corps. The primary goal of the meeting was "to identify different ways in which wearable technologies can be applied to a variety of military purposes; specifically training, equipment maintenance and operations, among others" [161]. David Evans, program manager with the CBRN Defense Coordinating Office, stated that the real challenge of wearables was not the availability of platforms but rather "refining the kind of data analysis that is useful for supporting decision making" [161].

As Shang notes, miniaturization plays a critical role in facilitating the continued successful development and design of WHDs, and "nanotechnology is a materials approach with the potential to facilitate device miniaturization while enhancing sensor performance" leading to larger sensitivity, quicker responses, and higher catalytic loadings [162]. Raza also notes that "in recent years, emerging conductive nanomaterials have been studied and used to develop wearable electrochemical biosensors due to characteristics like large specific surface area, high porosity, high sensitivity, and selectivity" [163]. Additionally, smaller sensors and smaller devices are improving comfort along with functionality allowing devices to be worn for extended periods of time with minimal discomfort [162]. In 2016, Oregon State University developed a contact lens with a nanostructured transistor made of amorphous indium gallium oxide that can detect glucose changes in tears [164]. Google also has a contact lens under development, although not much is publicly available about the technology at the time of writing.

6.3 IMPLANTABLE NANOSENSORS

Implantable sensors are those types of sensors that are embedded into tissues and can monitor physiological processes and biomarkers through direct integration with biological interfaces, such as cell membranes. Developments in engineering at the nanoscale have enabled better alignment of the bionanointerface, making this technology much more viable. Shang et al. [162] explained how nanofabricated devices could reduce or eliminate adverse responses to foreign devices, leading to more efficient calibration and longer operating times. Continuous glucose monitoring used by individuals with Type 1 diabetes is a current example of how implantable sensors are being used in healthcare [162, 165]. One system under development by Brolis (Vilnius, Lithuania) is the "spectrometer-on-a-chip" [166]. According to the company, the device uses an integrated sensor inside a device based on swept-wavelength infrared laser absorption spectroscopy to detect multiple analytes, including glucose, lactate, and ethanol through the skin (e.g., a fingertip).

One of the most advanced sensors for continuous monitoring is being developed by Profusa (Emeryville, CA), which has received funding from

DARPA in the past. The company claims these sensors are soft, flexible fibers ~5 mm long and 500 µm in diameter that offer continuous monitoring of body chemistries without causing inflammation or any localized rejection at the site [167]. Profusa's first commercially available offering is Lumee, an oxygen-monitoring platform used to measure oxygen levels in tissues, especially those affected by peripheral artery disease, chronic wounds, or following reconstructive surgery [168]. Lumee is CE marked for use in the European Union and has received investigational use in the United States. Designs such as Profusa's are not only important for biomonitoring but will also play a large role in monitoring the healing process of wounds, as Javaid et al. [158] noted:

> *"Monitoring the intertissued biological activities is vital during the manufacturing process utilizing regenerative medicine and tissue engineering methods. Such surveillance must be non-invasive to support essential tissue processes, including angiogenesis and cell signaling...The development, function, and concentration of essential minerals and gases, such as oxygen, should be considered."*

Although micro- and nanoelectronic technologies can drive down costs, improvement, and expansion of these devices into commercial markets, it will first be important to continue understanding more about the bionanointerface [162].

6.4 STRETCHABLE SENSORS AND FLEXIBLE ELECTRONICS

One of the most important and potentially revolutionary aspects of using engineered nanomaterials in sensor design is the ability to create sensors that can bend and flex. This is leading to sensors that move more naturally with human motion and are better able to produce efficient and life-like biomonitoring. Silicon (Si) is the most used substrate for many types of sensors and electronics, including on the micro- and nanoscale. However, Si-based platforms are limited by flexibility, temperature dependence, low signal, high noise, cost, and nonbiocompatible behavior [155]. These traits are essential for biomedical applications, hence the need and interest in exploring new materials for substrates and other components. This has led to research utilizing a wide range of materials (both nano- and macroscale). Regarding nanomaterials to achieve more flexible sensors, some commonly explored materials are CNTs, GR, carbon black (CB), and Au. Table 6-2 lists some of the types of nanomaterials that have been recently explored for various applications.

Raza et al. [163] provided an elegant overview regarding the use of stretchable nanomaterials in functional biomedical applications:

> *"The inherent properties of multidimensional nanomaterials, such as stretchability, provide excellent stability to sensors, which is essential for wearable applications. Furthermore, the porous structure of nanomaterials provides excellent immobilization for enzymes, thereby effectively increasing the diffusion of both the target and electrolyte, advancing the catalysis for the analyte. These nanomaterial sensing properties improve the performance and design strategies of wearable electrochemical biosensors. Conductive nanomaterials, particularly polymers, stand out as clear frontrunners, with significant advantages in explicit contact surface area, filler content, and operation electron transfer ratio."*

Stretchable sensors and electronics work well for applications that require constant direct contact with skin (or that need to move freely inside the body). Activities such as monitoring electrical signals or analytes in sweat are good examples. Lee and Hu have both developed conductive electrodes using nanomaterial to monitor ECG activity [169]. Both groups acknowledge that these

Table 6-2. GR/GO/CNT-Based, Flexible Sensing Devices *(Source: Das [160])*

No.	Sensor Type/Name of Structure	Materials	Flexibility Test Characteristics	Applications
1.	Chemical detection and actuation	Graphene	Stretchable up to 1500% strain for 500 cycles	Selective barriers, membranes, stretchable electronics, or soft robotics
2.	Multistimulus actuator	GO and CNT/PDMS	Cyclic stability up to 400 cycles with maximum bending up to 90°	Simulated biomimetic fingers, smart "tweezers," and humidity control switches
3.	NO_2 sensor	Ag NPs-decorated with rGO	Stable up to 3,000 bending cycles	Real-time monitoring of toxic gases in personal mobile electronics and human-machine interactions
4.	Strain sensor	Wrinkled graphene	Stretchability up to 300% strain	Monitor stretching deformation and human motion
5.	Transparent conductive films	SWCNT	No conductance degradation up to 4,000 bending cycles	Transparent electrodes in various flexible electronics
6.	Flexible transparent conductors	AgNW and SWCNT	Stable electrical properties up to 1,000 cycles	High-energy density, flexible-solid-state supercapacitors
7.	Flexible diode-transistor logic (DTL) driving circuit	SWCNT	Stable electrical properties up to 2,000 bending cycles	Drive and control quantum light emitting diode (QLED)
8.	Integrated wearable sensor	MOF/MWCNT	Stable electrical properties for bending angles up to 120°	Gas sensing and advanced wearable electronics for safety and healthcare purposes
9.	Flexible photodetector	MoS_2	Stable photoresponse characteristics up to 1,000 bending cycles	Flexible electronic devices
10.	Chemical vapor sensor	PANI/MoS_2	No changes in sensing performance after 500 cycles of stretching at 30% strain	Real-time monitoring of environment safety and human healthcare
11.	Visible light photodetector	BiI_3 nanoplates	Stable electrical response at 60° bending angle up to 200 cycles	Flexible 2-D optoelectronic devices
12.	Piezoresistive pressure sensor	MXene@CS@PU sheets	Stable output up to 5,000 cycles of compression and release	Detection of human physiological signals

sensors can do more, such as monitor analytes in sweat and record temperature. Table 6-3 identifies other uses or nanomaterials to create stretchable sensors.

6.5 FABRICS, TEXTILES, AND GARMENTS

Advancements in nanomaterial engineering and design have enabled the creation of fibers and wires that can be used as sensors or be integrated into other structures. A common trend is to weave sensing nanofibers and nanowires into textiles or create complete swatches of these nanosensing textiles and make garments from them, creating an entirely new class of "device" known as a smart fabric or smart textile. Recent examples are smart garments designed by Legionarius. These garments are loaded with biosensors that have the capacity to communicate a variety of information back to field medical personnel via constant relay through the Battlefield Assisted Trauma Distributed Observation Kit [170, 171]. The Legionarius garments can notify medical personnel when a service member has received a penetrating wound and can even apply pressure at the site to minimize or stop hemorrhage.

Ongoing work continues to demonstrate the feasibility of using nanofibers to create smart textiles for biosensing. In 2018, researchers from the Advanced Functional Fabrics of

Table 6-3. Materials Utilized in the Fabrication of Stretchable Strain Sensors *(Source: Bird [155])*

Materials	Type of Sensor	Stretchability (%)
CNTs-Ecoflex	Resistive	500
Aligned CNTs-PDMS	Resistive	280
CNTs-Ecoflex	Capacitive	150
CNTs-Dragon-Skin Elastomer	Capacitive	300
Graphene Foam-PDMS	Resistive	70
CBs-Thermoplastic Elastomer (TPE)	Resistive	80
Graphene-Rubber	Resistive	800
AgNWs-PDMS	Resistive	70
CBs-PDMS	Resistive	30
Zinc Oxide (ZnO) NWs-PDMS	Resistive	50
CBs-PDMS	Resistive	10
CBs-Ecoflex	Resistive	400
CNTs-Silicone Elastomer	Capacitive	100
AgNWs-Ecoflex	Capacitive	50
Platinum (Pt)-PDMS	Resistive	2
AuNWs-PANI-Rubber	Resistive	149.6
AgNWs-PEDOT: PSS/PU	Resistive	100
AuNWs-Latex Rubber	Resistive	350
CNTs-PEDOT: PSS/PU	Resistive	100

America, MIT Lincoln Laboratory, Inman Mills, and MIT embedded light-emitting diodes and photodetecting diodes into fibers [172]. These could be woven into fabrics where changes in light intensity can be used to determine a wearer's heart rate. The fibers are already being considered for integration into military uniforms. Researchers at MIT and in Sweden developed a new kind of fiber that can be made into clothing and can sense how much it is being compressed and stretch and provides pressure or vibration. These new fibers (OmniFibers) could be used to help patients recovering form respiratory issues but could also potentially be used to apply pressure in traumatic injuries for hemorrhage control [173]. Finally, research funded in part by the U.S. Army Research Office through the Institute for Soldier Nanotechnologies supported research that led to the design of a fabric that "works like a microphone, converting sound first into mechanical vibrations, then into electrical signals" [174]. When a single fiber was stitched into a shirt over the chest area, it accurately detected the heartbeat of a volunteer.

6.6 EMERGING TRENDS

6.6.1 Nanogenerators

A developing trend in nanobiosensing development is to design nanosensors that use environmental stimuli and physical forces to generate their own power. Piezoelectric nanogenerators

(PENGs), which generate electricity by applying mechanical pressure to a solid material and triboelectric nanogenerators (TENGs), which create an electrical charge via friction between two nonconductive surfaces, are two of the leading design types for sensors in this area. PENGs offer several advantages, including large availability of piezoelectric materials and composites; ability to harvest energy from low-intensity, low-frequency, and wide-ranging mechanical movements; ability to array the nanogenerators over large surface areas; and the ability to achieve scalable films [157]. TENGs can also be manufactured using a wide variety of materials, but unlike PENGs, they can be worn as flexible on-body sensors or woven into smart textiles. This makes them highly suitable for continuous health status monitoring, especially for real-time, sweat-based biosensing, due to their ability to produce constant power for continuous monitoring. Further, these require a simple and low-cost fabrication process, which makes them suitable for powering wearable medical devices and portable POC health monitoring systems, especially in resource-challenged settings. Bhide et al. [157] described several recent designs for self-powering PENGs and TENGs.

6.6.2 Sweat and Interstitial Fluid

There has been an interest in recent years to develop sensors that can detect analytes in fluids other than blood or saliva, especially using sweat or interstitial fluid. While some research exists in this area, few viable technologies, especially those using nanobased solutions, exist commercially. Chung et al. [175], Xu et al. [176], and Bhide et al. [157] described some of this research in much more detail. Although some of these technologies are still in their infancy, Bird et al. stated that "next generation bioelectronic sensors can be used to measure multiple signals such as heartbeat and metabolite secretion in perspiration, providing remote monitoring of the Warfighter's medical status during operations" [155].

6.6.3 Additive Manufacturing (AM)

AM (sometimes known as 3-D printing) is an emerging area of fabricating biosensors because this technique allows for more functionality to be packed into less volume. Nanomaterials are the primary choice in functionalizing thermoplastics or photocurable resins into diversified and functionalized analytical devices and sensors because of their thermostability, physiochemical properties and reactivity. This opens the possibilities for printing new active electronics in unique, functional, interwoven technology such as functionalized nanomaterials dispersed in solution-processable inks, which can be integrated into micron-scale coating or printing processes for the next-generation bioelectric sensors. Control over the deposition of nanomaterials dictates the performance of advanced printed devices, but the complex and dynamic forces involved in the drying process of the nanomaterial solutions are far from fully understood. Multiscale characterization and imaging of nanomaterial deposition help to elucidate the relationship between solvent evaporation and microstructure morphology [155].

6.7 CHALLENGES

Advanced technologies for medical monitoring have been in development for decades, and those using nanoenabled materials and manufacturing techniques have been explored for the past decade or so. However, several challenges remain before these technologies can reach full viability in commercial markets.

6.7.1 Funding and Innovation

One of the key drivers and limitation of nanosensor development is the funding and innovation policy. Fadel et al. [177] noted that although there has been significant progress made in nanosensor development, the "valley of death" gap still plagues much research in this area. Much of this problem stems from the ability of nanotechnology devices to have high scalability and low production. Das et al. [160] explained that this can be mitigated through better integration of different areas

(printed electronics, fabrication technology, and nanomaterial science) as well as developing devices with reliable power sources and better data management software packages.

6.7.2 Privacy and Security

Not necessarily a technical challenge, but certainly important, are the privacy and security issues associated with sensing devices. Recently, an issue arose with U.S. military personnel using an app called Strava. Strava is a mobile device app that synchronizes with a wearable device and provides metrics on running. However, it was found that the app also tracked runners' locations through GPS, and accessing the app showed the perimeter of the base where personnel were stationed [178]. Additionally, there have been privacy and ethical concerns associated with implantable nanosensors. Continuous monitoring offers great benefits to health but also opens the door for accessibility issues to health data [179].

6.7.3 Materials Limitations

Carbon-based nanomaterials have been used quite extensively to make sensing electrodes, especially for detecting metabolites like glucose; but these materials cannot catalyze glucose directly and need to be modified [163]. This enzymatic inactivation decreases the lifespan of glucose electrochemical sensors and is a key limit to commercialization. CNTs are difficult to tune and continue to have purity issues, and GR has restricted stretchability and is prone to developing unrepairable cracks during stretching cycles [160]. Metal nanomaterials can detect glucose much better than carbon-based nanomaterials but are still expensive to manufacture and can corrode and lose conductivity over time [163]. Conductive polymer nanomaterials are commonly explored for sensors as well but do not have good catalytic properties like metal-based nanomaterials [163].

SECTION 07 CONCLUSION

The use of emerging and advanced technologies in CCC is not new. Much of the equipment and many techniques used in civilian trauma care were developed out of necessity (or greatly improved upon) on the battlefield (i.e., the tourniquet, the ambulance, helicopter-based medical evacuations, etc.). Much of this initial ingenuity was also made possible by the availability of new technologies and a willingness to explore their uses in medical care. The DoD has unique challenges to providing medical care in austere and dangerous environments. While principles of trauma care and medicine are still followed in the field, these daunting situations often provoke a need to implement grand solutions to address emerging and persistent challenges not faced anywhere else in civilian sectors. Technologies, especially new types, are often explored as vehicles to mitigate these new challenges. One such technology is nanotechnology, as discussed in this report.

Nanotechnology research evolved during the development of current CCC guidelines. It has been present throughout the past 20 years of war, where we advanced medical research and developed applications necessary to treat new patterns of injuries from new weapons. One of the turning points in nanotechnology R&D, especially in the United States, was the creation of the National Nanotechnology Initiative in 2000 [180]. One year later, the United States was attacked on September 11, 2001, leading to 20 years of war in Iraq and Afghanistan. The first lethal improvised explosive device (IED) attack on American forces occurred in 2003. It was the beginning of what would become the leading weapon of an insurgency. IED attacks caused the defining injury of the war—TBI—and left a distinct injury pattern of polytrauma. The DoD had to pivot and adapt to treating extremely dirty, severe limb and body wounds that affected multiple tissue types as well as head injuries. Many of the applications of nanotechnology in CCC have mirrored the needs of field medical personnel over the past 20 years. However, now that nanotechnology is maturing, how can it be used for treating injuries of future wars?

In an era of future battlefields and MDOs, new threats, like directed energy weapons and kinetic weapons, are expected to play a larger role in combat. These will create new types of injuries and patterns of injury for U.S. forces and their allies. A return to combat with more traditional adversaries and forces will see the use of older weapon types, including artillery, which U.S. forces have not faced since the Korean War. Additionally, there is an increasing threat of biological, chemical, and/or radiological/nuclear weapons. Some of these are becoming much more sophisticated and can complicate a battlefield littered with trauma injuries. The DoD R&D enterprise is in prime position to leverage two things: (1) the lessons learned from treating combat injuries of the past and how best to leverage those lessons for future operations and (2) an extremely robust portfolio of technology solutions that can be adapted and used for any type of medical scenario. This requires a balance, as the focus should be on treating injured

Warfighters with well-trained people who are given the best tools to complete that mission.

Nanotechnology on its own is a multidisciplinary field with roots dating to the late 1950s but rapidly expanding over the past 20 years. The field has made inroads into almost every area of science and engineering, and medicine is no exception. Dubbed nanomedicine, the use of nanotechnology to address medical needs has been a hot topic, prompting the development of programs not just in education but also in R&D and clinical practice. The DoD has been exploring applications of nanotechnology since the late 1990s and spends millions of dollars annually on medical research. The Department has also been exploring ways to take advantage of nanomedical applications, especially for CCC, given the advantages that technology often brings to field medical care.

In this report, we explored five areas deemed inclusive of CCC overall, based on a review of the structures of relevant DoD agencies and areas of research (Figure 7-1).

We also identified two overall aspects of the use of nanotechnology in CCC. First, existing types of applications that have been used for years are being made smaller due to the introduction of nanotechnology manufacturing methods and materials. Miniaturization of some of these technologies is indeed opening new possibilities, such as the use of nanosensors for biological monitoring in real-time or the development of better wound dressings. Second, nanotechnology is creating something entirely new and novel and poised to revolutionize one or more of the five areas in this report, creating new and unique solutions. One possible application would be the development of scaffolds for tissue regeneration or the construction of lipid NPs for drug delivery. Overall, we did find that nanotechnology enabled movement in some areas of CCC—some more than others. General directions of nanotechnology research and application in CCC follow these patterns:

- **Hemorrhage Control and Fluid Resuscitation** – clearly leading to solutions in artificial whole blood.
- **Wound Management** – shift from passive barriers to more interactive and bioactive style dressings.
- **Neurotrauma and Pain Control** – rapid diagnostics of brain injuries at time of impact and quicker pain relief solutions.
- **Tissue Engineering** – bone regeneration.
- **Advanced Medical Monitoring and Diagnostics** – moving the POC to the individual and extending capabilities of field medical personnel through a combination of real-time monitoring and theragnostics.

This research revealed some clear trends and themes that weave throughout the entire practice of nanotechnology in CCC. First, we have seen the ubiquitous and growing interest of using chitosan

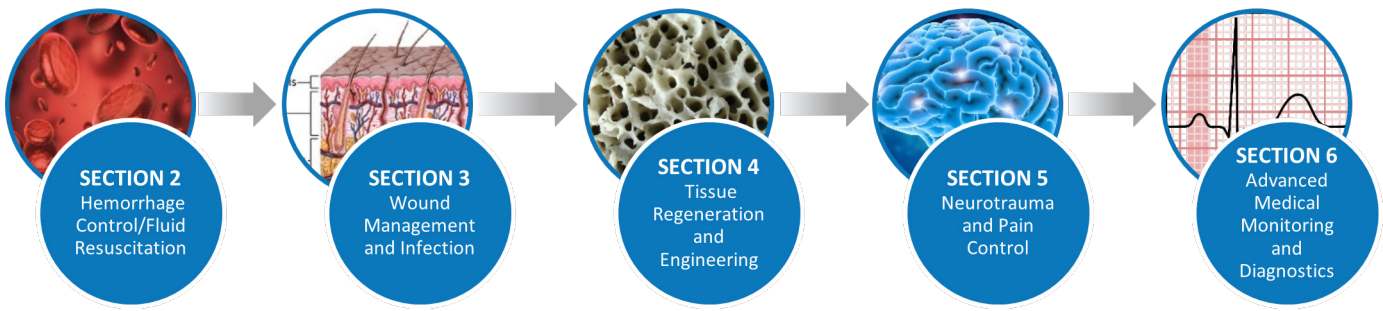

Figure 7-1. Five Core Content Areas of the Report and an Illustration of the Flow of Content *(Source: Greg Nichols).*

for all types of medical applications, from bandages to tissue engineering to fighting infection. Next, the expansion of lipid NPs as carriers for all types of therapeutics, from antibiotics to siRNA, is pervading almost all areas explored herein. Furthermore, nanotechnology is not just a standalone concept anymore but is overlapping with other emerging technologies, including biotechnology, 3-D printing, and internet of things/sensors. This contributes to the concept known as convergence, where the borders of multiple fields combine and become blurred. Additionally, nanotechnology and engineered nanomaterials are being subsumed into the broader category of advanced materials, which will, in many ways, further diversify and expand nanotechnology research. Finally, through a combination of the diversity and utility of nanotechnology itself and the trend towards convergence, the areas explored in this report are slowly overlapping more into a menagerie of a more unified field, still diverse but with undertones of familiarity and shared relevance. In many ways, nanomedicine is showing a path forward for how new tools and techniques can be shared for a unified purpose.

Nanotechnology, as it pertains to CCC and what the DoD is doing in this area, is one piece of this report. The other aspects are how relevant research in other fields and organizations can feed their lessons learned back into the needs of acute trauma care and how many other relevant concepts and areas spin off this. For example, R&D in nanomedicine applications for oncology (antineoplastic agent delivery), neurology (AD and PD treatment), diabetes care (continuous glucose monitoring and chronic wound care), and orthopedics (tissue repair and infection control) have used the boundaries of what is possible in hospital-based and long-term care. How can advances in these fields be transitioned to prehospital care and CCC?

Similarly, R&D conducted in nanotechnology and elsewhere by agencies outside the DoD may soon have direct applicability to CCC. Some of this was mentioned in this report, but the increased interest of using advanced materials and advanced technologies in medicine is rapidly expanding and being conducted across a wide range of institutions. As an illustration, the DoD should consider how to take advantage of synergies from relevant research occurring at other agencies, such as the following:

- **DOE** – advanced materials and AM capabilities, biomedical research.
- **NASA** – medical care in remote and austere environments.
- **VA** – continuation of DoD-related medical care (long-term care).
- **U.S. Department of Health and Human Services (Biomedical Advanced Research and Development Authority)** – advanced technologies to treat acute injuries.
- **U.S. Department of Homeland Security** – programs to support first responders.

Technological trends in medicine not only apply to nanotechnology but also to the solutions interfacing with nanotechnology. The incorporation of nanotechnology by the DoD into CCC could set up a roadmap for other technological areas in CCC being explored by the DoD. These include the following:

- Artificial intelligence.
- 3-D printing/four-dimensional printing.
- Internet of things/sensors/connected devices.
- Autonomy.
- Robotics.
- Biotechnology/synthetic biology.
- Other advanced materials.

Grand challenges to further adopt nanotechnology in CCC are nearly identical to the challenges seen in other fields—regulations that lag and may not clearly meet current technological needs, health and safety considerations, and privacy and data collection standards for wearables and

nanosensors. At one point in time, nanotechnology was so new and research funding was so available that nanoenabled solutions were prized over other solutions. This was important, and still is, to fully explore what it possible or could be possible to build at this scale. The field pushed the limits of what was possible but also strayed at times from what was needed.

Although there may have been a change in thinking over the past several years that emphasizes practical solutions in general, nanotechnology-enabled products are still very much in the mix. Many of these new products still mimic nanoenabled ones since more advanced medical applications demand more sophisticated solutions, oftentimes at the nanoscale. However, as the emphasis shifts focus toward more general to advanced materials and practical biomedical designs, funding agencies and program managers are less likely to explicitly ask for solutions at the nanoscale. Even though these types of solutions will still be expected and submitted, there is a risk of not specifically including wanted solutions at the nanoscale. Direct and consistent funding for research in this domain is important. As peer and near-peer competitors continue to develop advanced biomedical solutions, a careful balance must be struck that continues to promote the continued development of nanoenabled medical applications reasonably and realistically for CCC without being overly stuck on nanotechnology alone.

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