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Review Article

Impact of nutritional factors on colorectal cancer: implication The molecular mechanisms of vitamins supplementation

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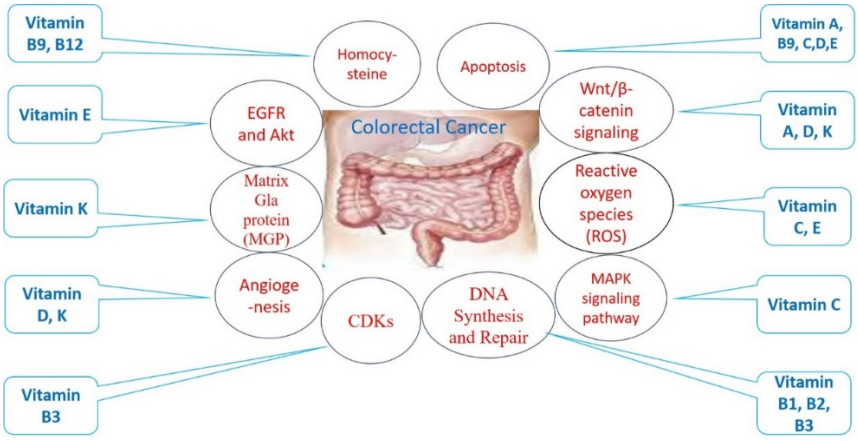
Abstract

Colorectal cancer (CRC) is the leading cause of death from gastrointestinal cancer, the second largest cause of cancer-related death worldwide, and the third most common cancer in both men and women. Bad eating habits, smoking, intestinal inflammatory diseases, polyps, aging, and genetic factors all increase the risk of developing colorectal cancer. More interestingly, vitamin deficiency has been associated with an increased risk of colorectal cancer. We reviewed the published significance of vitamin supplementation on colorectal cancer proliferation, survival, apoptosis, angiogenesis and migration, focusing on the possible molecular mechanisms of water-soluble and fat-soluble vitamins to provide protective suggestions to minimize the occurrence and progression of colorectal cancer.

Keywords: colorectal cancer; nutritional factors; vitamins supplementation



Graphical abstract of the possible molecular mechanisms of vitamins on colorectal cancer.

Introduction

One of the most prevalent cancers in the world is colorectal cancer. It ranks second in terms of cancer-related deaths and third in terms of incidence [1]. In 2018, there were 1.8 million new cases of colorectal cancer worldwide, accounting for nearly 10% of all new cancer cases and deaths worldwide [2]. By 2040, it is predicted that there will be over 1.9 million new cases of colorectal cancer, making it the third most diagnosed cancer worldwide [3]. There could be nearly 2.5 million new cases in 2035. According to data from the USA, the death rate decreased by approximately 50% between 1970 and 2016, from 29.2 per 10,000 patients to 13.7 per 10,000 patients, due to the rapid advancements in screening and treatment procedures. However, this tendency seems only observed in highly developed countries [4].

Meanwhile, the 5-year survival rate for colorectal cancer is roughly 64%, whereas the rate for metastatic colorectal cancer is 12%. Further research is still required to develop effective medical

intervention strategies [5]. The main topic of this review is the role of vitamins in preventing and protecting against colorectal cancer. Moreover, a comprehensive understanding of the underlying molecular mechanisms of vitamin-mediated epigenetic regulation of colorectal cancer genes is required to target therapeutic targets for colorectal cancer prevention and treatment effectively.

Vitamin A

Vitamin A is essential for many physiological functions, such as cellular differentiation regulation and epithelial tissue health maintenance. Numerous molecular mechanisms have been identified as potential means by which vitamin A and its derivatives, including Retinoids, may impact the development of colorectal cancer. Moreover, *Wang* and his colleagues had stated that vitamin E, C, and carotenoid intake did not correlate with the risk of colorectal cancer in either men or women [6].

Vitamin A is converted into its active form, retinoic acid, which binds to nuclear receptors, such as retinoid X receptors (RXRs) and retinoic acid receptors (RARs) [7]. These receptors control gene transcription, affecting how epithelial cells differentiate. Thus, proper cell differentiation is crucial for maintaining the typical architecture and function of the colorectal epithelium. Dysregulation of this process can contribute to carcinogenesis [8]. Moreover, it has been demonstrated that retinoid affects the expression of cell cycle regulators, which modulate the cell cycle. They can stop cell division and induce cell cycle arrest, which stops the unchecked growth linked to cancer [9]. Furthermore, it has been reported that colorectal cancer cells can undergo apoptosis when exposed to vitamin A and its derivatives [9,10]. This process of programmed cell death removes harmed or aberrant cells, serving as a defence against cancer growth.

Retinoids play a role in modulating the immune system, potentially enhancing the body's ability to recognize and eliminate cancer cells. This includes effects on immune cell function and the production of cytokines [11], as it has been reported that the retinoic acid receptor in the nucleus of bone marrow cells is most likely bound by vitamin A, which controls the population of bone marrow cells. This, in turn, inhibits the expression levels of apoptosis genes, including B-cell lymphoma 2 (Bcl-2) and Fas, where Bcl-2 interfere with apoptosis processing by delaying Fas-induced apoptosis and caspase activation [12]. Furthermore, retinoids may inhibit angiogenesis, the process of creating new blood vessels, which is essential for the growth of tumours. By limiting the blood supply to tumours, Retinoids can suppress tumour progression [13].

The Wnt/Wingless signaling transduction pathway is involved in the development of embryos as well as tumorigenesis. The transcription of Wnt target genes is activated by β-Catenin, a crucial element of the Wnt signaling pathway, through its interaction with the TCF/LEF transcription factor family [14]. *Bian* and his colleagues, demonstrated that The Wnt/β-catenin signaling pathway is thought to be abnormally activated in colorectal cancer because almost all colorectal cancer patients have increased Wnt/β-catenin signaling, which emphasizes the significance of this pathway for therapeutic intervention [15]. Retinoids have been shown to interact with this pathway, influencing the expression of Wnt target genes and preventing aberrant cellular reactions linked to the emergence of cancer [9,16].

Moreover, vitamin A and its derivatives can influence epigenetic modifications, such as DNA methylation and histone acetylation [17]. These epigenetic changes can alter gene expression in colorectal cancer development. In addition to DNA methylation, miRNA expression, and genomic imprinting, histone modification is becoming more widely acknowledged as a crucial mechanism behind the onset and progression of colorectal cancer [18].

Vitamin B1 (Thiamine)

The specific molecular mechanisms underlying the effects of vitamin B1 (thiamine) on colorectal cancer are not as extensively studied and understood as those for some other vitamins. Thiamine is an essential B vitamin critical in energy metabolism, particularly in converting glucose to energy [19].

Thiamine is a cofactor for enzymes involved in the metabolism of carbohydrates, particularly in the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway [19]. Alterations in energy

metabolism are a hallmark of cancer, and maintaining proper energy balance is crucial for normal cell function. Imbalances in energy metabolism could potentially affect the growth and survival of colorectal cancer cells [20].

Thiamine participates in the regeneration of the antioxidant glutathione [21]. Antioxidants protect cells from oxidative stress, which is implicated in cancer development. By contributing to the cellular antioxidant defense system, thiamine may indirectly influence cells' susceptibility to oxidative damage.

Thiamine is involved in nucleotide synthesis, essential for DNA stability and repair. Appropriate mechanisms for DNA repair are also essential for avoiding the build-up of mutations that may aid in the development of cancer [22]. B vitamins, including thiamine, play a role in supporting immune system function. A well-functioning immune system is critical for recognizing and eliminating cancer cells. Thiamine deficiency has been associated with immune system dysfunction, and restoring thiamine levels could potentially support immune responses against colorectal cancer [23].

Vitamin B2 (Riboflavin)

Riboflavin, a water-soluble vitamin B2, is a precursor to the cofactors flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which are necessary for many cellular functions, such as energy metabolism [24].

Riboflavin is a critical component of the electron transport chain, where it participates in oxidative phosphorylation within the mitochondria. Proper mitochondrial function is essential for cellular energy production [25]. Dysregulation of energy metabolism is a common feature of cancer cells, and riboflavin's role in this process could indirectly impact colorectal cancer cells [26].

Riboflavin synthesizes the antioxidant cofactors FMN and FAD, which are essential for the activity of antioxidant enzymes [24,25]. Antioxidants help protect cells from oxidative stress, and maintaining an adequate supply of riboflavin may contribute to cellular defence mechanisms against oxidative damage, which is associated with cancer development [27].

Riboflavin is involved in nucleotide metabolism, crucial for DNA synthesis and repair [28]. Proper DNA repair mechanisms are crucial to stopping the accumulation of mutations that might lead to the development of colorectal cancer [29].

Chronic inflammation is a known risk factor for cancer development, including colorectal cancer [30]. Some B vitamins, including riboflavin, may have anti-inflammatory effects, potentially contributing to a lower risk of cancer [31]. Riboflavin is essential for the normal functioning of the immune system [32]. A well-functioning immune system is crucial for recognizing and eliminating cancer cells. Adequate riboflavin levels may support immune responses against colorectal cancer [32].

Vitamin B3 (Niacin)

Vitamin B3, also known as niacin, is a water-soluble vitamin that plays essential roles in cellular metabolism and DNA repair. The effects of vitamin B3 on colorectal cancer involve several molecular mechanisms [33]. Nicotinamide adenine dinucleotide (NAD+) is a coenzyme involved in energy metabolism, DNA repair, and cell signaling, as well as Niacin is a precursor for NAD+. For cellular health and function to be maintained, adequate levels of NAD+ are essential [34]. Moreover, NAD+ is a cofactor for several enzymes involved in energy metabolism, such as those in the glycolytic pathway and the tricarboxylic acid (TCA) cycle [35].

Also, Poly (ADP-ribose) polymerase (PARP) enzymes, which utilize NAD+ as a substrate, play a role in DNA repair [36]. Niacin supplementation may impact DNA repair processes, contributing to genomic stability and reducing the risk of mutations leading to colorectal cancer. Moreover, NAD+-dependent sirtuin enzymes have been implicated in regulating angiogenesis and blood vessel formation [37]. Tumor growth requires proper control of angiogenesis, and niacin's effect on sirtuin activity may impact this process [38]. Most interestingly, it has been proposed that niacin affects the function of cyclin-dependent kinases (CDKs), which control the cell cycle [39].

Vitamin B5 (Pantothenic acid)

Vitamin B5 is an essential water-soluble vitamin that is a coenzyme A (CoA) component, which plays a critical role in various cellular processes, including energy metabolism and the synthesis of fatty acids and cholesterol. Pantothenic acid is a precursor to CoA, which is essential for acetyl-CoA synthesis. Acetyl-CoA is a critical intermediate in energy metabolism, linking the breakdown of carbohydrates, fats, and proteins to producing ATP, the cell's primary energy source. Dysregulation of energy metabolism is a hallmark of cancer, and alterations in these pathways could impact colorectal cancer cells [40].

More interestingly, CoA is involved in various mitochondrial reactions, maintaining cellular redox balance [41]. Moreover, vitamin B5's role in supporting mitochondrial function may affect colorectal cancer. Furthermore, Acetyl-CoA, generated from vitamin B5-derived CoA, is involved in acetylation reactions that regulate gene expression and cellular signaling [42]. Aberrant acetylation patterns have been observed in cancer cells, and Vitamin B5 may indirectly influence these processes [41,42].

Vitamin B6 (Pyridoxine)

Vitamin B6 is a water-soluble vitamin in several forms, including pyridoxine, pyridoxal, and pyridoxamine. The active coenzyme forms of vitamin B6, pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP) play crucial roles in various biological processes [43]. Vitamin B6 is involved in one-carbon metabolism, including converting homocysteine to cysteine [44]. The regulation of homocysteine levels is essential for DNA methylation and synthesis, and disturbances in this process have been associated with cancer, including colorectal cancer [45].

Moreover**,** PLP, the active form of vitamin B6, is a cofactor for various enzymes involved in amino acid metabolism [46]. These enzymes synthesize and break amino acids, which are essential for cell growth and proliferation. Furthermore, PLP is a cofactor for enzymes involved in heme synthesis. Heme is an essential component of haemoglobin and other hemoproteins [47]. Alterations in heme metabolism may influence oxygen transport and cellular respiration, potentially impacting cancer cells.

More interestingly, PLP synthesizes neurotransmitters such as dopamine, serotonin, and gamma-aminobutyric acid (GABA). Neurotransmitters can influence cellular signaling, and disturbances in neurotransmitter balance may impact cancer progression [48].

Vitamin B7 (Biotin)

Vitamin B7, known as biotin, is a water-soluble cofactor for several carboxylase enzymes involved in various metabolic pathways [49]. Carboxylase enzymes, such as pyruvate carboxylase, acetyl-CoA carboxylase, and propionyl-CoA carboxylase, require biotin as a cofactor [50]. These enzymes play crucial roles in fatty acid synthesis, gluconeogenesis, and amino acid metabolism. Dysregulation of these metabolic pathways has been linked to cancer, and biotin's role in supporting carboxylase function may indirectly impact these processes. Moreover, Biotin can modulate gene expression, and some studies suggest it may play a role in cell proliferation and growth [51]. Uncontrolled cell proliferation is a hallmark of cancer, and factors that regulate cell cycle progression are of interest in cancer research.

Biotin has been suggested to modulate immune responses, and a well-functioning immune system is critical for recognizing and eliminating cancer cells [52]. The influence of biotin on immune function may have implications for colorectal cancer.

Vitamin B9 (Folic acid)

Vitamin B9 also called folic acid, is the synthetic form of folate or folate, a water-soluble vitamin that plays a crucial role in various cellular processes, including DNA synthesis, repair, and methylation [53]. Folate is essential for normal cell function and development, and its status has been implicated in developing and preventing colorectal cancer [54]. Folate is involved in synthesising thymidylate, a DNA synthesis precursor. Thymidylate is necessary to produce thymine, one of the four nucleotide bases that make up DNA [55]. Adequate folate levels support normal DNA synthesis and repair, which is crucial

for preventing mutations that can lead to cancer [56]. Moreover, folate is a crucial donor of methyl groups essential for DNA methylation. DNA methylation is an epigenetic modification that regulates gene expression [57].

Most interestingly, Folate, along with vitamins B6 and B12, is involved in converting homocysteine to methionine [58]. Elevated levels of homocysteine are associated with an increased risk of colorectal cancer, and folate supplementation has been shown to help lower homocysteine levels [59]. Folate plays a role in regulating cell proliferation and apoptosis (programmed cell death). Adequate folate levels are necessary for maintaining a balance between cell growth and cell death. Dysregulation of these processes can contribute to cancer development [60]. Folate deficiency has been linked to chromosomal instability, a hallmark of cancer. Adequate folate levels are essential for maintaining chromosomal integrity and preventing structural abnormalities that can contribute to cancer development [61].

Moreover, folate status may influence the expression of microRNAs, small RNA molecules that play a role in post-transcriptional gene regulation. Changes in microRNA expression patterns have been observed in colorectal cancer [62]. Furthermore, folate supplementation has been associated with reducing DNA strand breaks, providing protection against DNA damage [63]. This is important for preventing the accumulation of genetic alterations that can contribute to colorectal cancer.

Vitamin B12 (Cobalamin)

Cobalamin, another name for vitamin B12, is a water-soluble vitamin essential for many cellular functions, such as DNA synthesis, red blood cell production, and neurological function [64]. Vitamin B12 is involved in the conversion of homocysteine to methionine, a process that also requires the participation of folate and vitamin B6. Methionine is crucial for synthesizing thymidylate, a nucleotide necessary for DNA replication and repair [65]. Most interestingly, vitamin B12 is involved in the methylation of DNA through its role in providing methyl groups for one-carbon metabolism [66]. Moreover, vitamin B12 is involved in the normal functioning of the immune system [67]. A well-functioning immune system is critical for recognizing and eliminating cancer cells. Adequate vitamin B12 levels may support immune responses against colorectal cancer [68].

Vitamin C (Ascorbic acid)

Vitamin C, or ascorbic acid, is a water-soluble vitamin with antioxidant properties. While its role in preventing and treating colorectal cancer is a topic of ongoing research, several potential molecular mechanisms through which vitamin C may influence it exist. Vitamin C is a potent antioxidant that can neutralize reactive oxygen species (ROS) and free radicals [69]. Colorectal cancer development is associated with oxidative stress, and the antioxidant properties of vitamin C may help reduce DNA damage and genomic instability [70]. Also, vitamin C is essential for collagen synthesis, which is crucial for maintaining the integrity of the extracellular matrix. The extracellular matrix plays a role in cell adhesion, migration, and invasion, which are relevant to cancer progression [71,72]. Moreover, vitamin C is involved in the function of immune cells, including lymphocytes and phagocytes. Adequate vitamin C levels may enhance immune response against colorectal cancer cells [73]. Most interestingly, vitamin C is a cofactor for dioxygenase enzymes, such as the ten-eleven translocation (TET) enzyme involved in DNA demethylation. DNA methylation patterns are altered in cancer, and vitamin C's role in epigenetic regulation may impact gene expression and cellular behaviour [74,75].

Vitamin C disrupts the mitogen-activated protein kinase (MAPK) signaling pathway, which is involved in cell division, survival, and apoptosis [76,77]. Modulation of these signaling pathways may impact the behaviour of colorectal cancer cells. Recent studies show that vitamin C has pro-oxidant effects in cancer cells, leading to selective cytotoxicity. This is thought to be due to the generation of hydrogen peroxide in the presence of transition metal ions [78]. Furthermore, vitamin C has been investigated for its potential to enhance the effectiveness of chemotherapy. Some studies suggest that vitamin C may sensitize colorectal cancer cells to the effects of specific chemotherapeutic agents [79,80].

Vitamin D (Cholecalciferol)

Vitamin D is a fat-soluble vitamin that plays a crucial role in calcium homeostasis, bone health, and immune function [81]. Emerging evidence suggests that vitamin D may also prevent colorectal cancer, as demonstrated in Figure 2 [82].

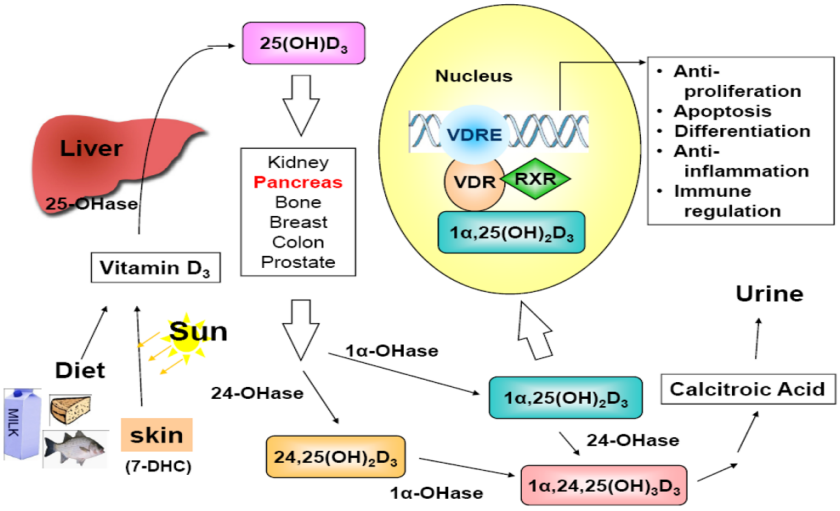


Figure 2. Vitamin D sources, metabolism, mechanism of action and biological activities [101].

Vitamin D can inhibit cell proliferation by inducing cell cycle arrest. It exerts its effects through interactions with the vitamin D receptor (VDR), leading to the regulation of genes involved in cell cycle control [83,84]. Dysregulation of cell cycle progression is a hallmark of cancer, and vitamin D's influence on this process may contribute to its anti-cancer effects [85,86]. Moreover, vitamin D's ability to promote apoptosis may help prevent cancer cell survival and proliferation [87]. It can also inhibit angiogenesis, forming new blood vessels. Inhibition of angiogenesis is considered a strategy to limit the blood supply to tumours, thereby restricting their growth and metastasis [88,89]. Also, vitamin D may enhance DNA repair mechanisms, contributing to the maintenance of genomic stability [90]. This is important for preventing the accumulation of mutations that can lead to the initiation and progression of colorectal cancer. Vitamin D can modulate immune responses by influencing the function of immune cells, including T cells and macrophages. A well-functioning immune system is critical for recognizing and eliminating cancer cells [91]. Furthermore, vitamin D exerts its biological effects by binding to the Vitamin D receptor (VDR). VDR activation leads to gene expression regulation, affecting various cellular processes. Alterations in VDR expression and function have been associated with colorectal cancer risk [92,93]. Vitamin D has been shown to downregulate the Wnt signaling pathway, which plays a crucial role in colorectal carcinogenesis. Dysregulation of Wnt signaling is common in colorectal cancer [94,95]. Also, several recent studies demonstrate that vitamin D has been shown to suppress the Wnt/β-catenin signaling pathway, a critical pathway involved in colorectal carcinogenesis. Vitamin D's inhibitory effects on this pathway may contribute to its anti-cancer properties [96-101].

Vitamin E (Tocopherol)

Vitamin E is a group of fat-soluble antioxidants, including tocopherols and tocotrienols, each with distinct molecular structures and potential health effects. Vitamin E, as an antioxidant, can neutralize ROS and free radicals [102]. ROS are implicated in DNA damage, inflammation, and cellular stress, all of which can contribute to cancer development. By scavenging ROS, vitamin E may help protect cells from oxidative damage. Most importantly, Vitamin E can influence various signalling pathways in cell growth, survival, and apoptosis. These pathways include those related to the epidermal growth factor receptor (EGFR) and protein kinase B (Akt), which play roles in colorectal cancer development [103-106]. Furthermore, vitamin E, especially alpha-tocopherol, is known for protecting cell membranes from lipid peroxidation. This preservation of cell membrane integrity may affect cellular function and survival [107].

Vitamin K

As a fat-soluble vitamin, vitamin K is essential for bone metabolism and blood clotting. Vitamin K comes in two primary forms: K1 (phylloquinone) and K2 (menaquinone) [108]. Vitamin K is essential for the gamma-carboxylation of specific proteins, including clotting factors involved in blood coagulation. Beyond coagulation, vitamin K-dependent proteins (VKDPs) play roles in processes such as bone metabolism and may have implications for cancer [109]. The Wnt signaling pathway is frequently dysregulated in colorectal cancer. Some studies suggest that vitamin K may modulate the Wnt signaling pathway, influencing cell differentiation, proliferation, and survival [110-112]. Moreover, vitamin K has demonstrated anti-angiogenic properties, which may inhibit the formation of new blood vessels that supply nutrients to tumours. Limiting angiogenesis can impede the growth and spread of colorectal cancer [113]. Moreover, Matrix Gla-protein (MGP) is a vitamin K-dependent protein inhibiting vascular calcification. The regulation of MGP may have implications for vascular health, which is relevant to colorectal cancer progression [114-118]. Moreover, vitamin K has been suggested to play a role in epigenetic regulation, potentially affecting gene expression patterns. Epigenetic changes are involved in cancer development, and vitamin K's impact on this process may contribute to its anti-cancer effects [119].

Colorectal cancer risk factors related to vitamin deficiency

Numerous studies have investigated the relationship between vitamin deficiency and colorectal cancer occurrence and progression. Vitamin D deficiency has been extensively studied for colorectal cancer. Several epidemiological studies have shown an inverse association between vitamin D levels and colorectal cancer risk [120]. Lower circulating levels of vitamin D have been associated with an increased risk of colorectal cancer incidence and mortality. Additionally, vitamin D deficiency has been implicated in colorectal cancer progression, including tumour growth, invasion, and metastasis [121]. Moreover, vitamin A and its derivatives, including retinoids, have been implicated in colorectal cancer prevention and progression. Animal studies have shown that vitamin A deficiency increases susceptibility to colorectal cancer development, while dietary supplementation with vitamin A or retinoids can inhibit tumour growth and progression [122]. Furthermore, studies examining the association between vitamin E levels and colorectal cancer risk have yielded mixed results. Some studies have reported an association between vitamin E intake or serum levels and colorectal cancer risk [123]. More research is needed to clarify the role of vitamin E in colorectal cancer prevention and progression. Vitamin C is a potent antioxidant that may protect against colorectal cancer by scavenging free radicals and reducing oxidative stress. Some epidemiological studies have suggested an inverse association between vitamin C intake or serum levels and colorectal cancer risk, although results have been inconsistent [124].

Moreover, vitamin C deficiency has been associated with colorectal cancer progression, including tumour growth and metastasis, possibly due to impaired antioxidant defence mechanisms and increased oxidative stress. Finally, vitamin K, particularly vitamin K2 (menaquinone), has been studied for its potential role in colorectal cancer prevention and treatment. Vitamin K deficiency has been associated with an increased risk of colorectal cancer incidence and mortality in some observational studies [125]. Additionally, preclinical studies have shown that vitamin K2 supplementation inhibits colorectal cancer cell proliferation, invasion, and metastasis, possibly by regulating cell signaling pathways, including the MAPK pathway [126].

Summary and Conclusion

The risk of colorectal cancer is increased by several environmental lifestyle factors that are primarily modifiable, including smoking, drinking too much alcohol, and gaining weight. More interestingly, there are some modifiable nutritional risk factors such as consuming red and processed meat, low fibre intake, low vitamin D level and consuming a high-fat diet; vitamin deficiency may increase the risk of colorectal cancer. In this review, we focus on the molecular mechanisms of vitamins on colorectal cancer tumorigenesis and progression and demonstrate, based on the previous studies, that all vitamin

supplementation may protect against colorectal cancer. However, some vitamins have precise molecular mechanisms on molecular pathways involved in cancer proliferation, survival, angiogenesis migration and metastasis, such as vitamins A, B3, B9, C, D, E and K.

Future directions

Examining the impact of nutritional factors, particularly vitamins, on colorectal cancer through molecular mechanisms presents a rich avenue for research. Thus, we suggest the following future directions:

- Further elucidating the molecular mechanisms by which vitamins influence colorectal cancer development and progression involves investigating their effects on key signaling pathways, such as the MAPK pathway, Wnt/β-catenin pathway, PI3K/AKT pathway, and NF-κB pathway, which are dysregulated in colorectal cancer.
- Identifying specific genes, proteins, and metabolites modulated by vitamins and their implications for colorectal cancer pathogenesis.
- Investigating the potential interactions and synergies between different vitamins and other dietary factors in colorectal cancer prevention and treatment. This involves examining how combinations of vitamins or vitamins with other bioactive compounds (e.g., phytochemicals, minerals) may exert additive or synergistic effects on colorectal cancer-related molecular pathways.
- Translating findings from preclinical studies into clinical settings by conducting well-designed clinical trials to evaluate the efficacy and safety of vitamin supplementation as adjuvant therapy or preventive intervention for colorectal cancer.
- Investigating the potential of nutritional interventions, including vitamin supplementation, in high-risk populations for colorectal cancer, such as individuals with a family history of colorectal cancer, inflammatory bowel disease (IBD), or genetic predisposition to colorectal cancer (e.g., Lynch syndrome). This involves assessing such interventions' feasibility, acceptability, and efficacy in real-world settings.
- Assessment of the long-term outcomes and survivorship benefits of vitamin supplementation in colorectal cancer patients, including recurrence rates, overall survival, and quality of life. This includes evaluating the impact of vitamin supplementation on colorectal cancer recurrence, treatment-related side effects, and comorbidities in long-term survivorship.

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None

Authors contribution

The two authors are fully accountable for expertly executing and crafting every aspect and component of this work.

Declaration of interest

The authors declare no conflict of interest.

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Review Article

Abdominal Wall Defects: Hydrogel based solutions in abdominal wall reconstruction

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Abstract

Abdominal wall defects, including hernias and congenital anomalies, are complex conditions that require practical and durable repair strategies. While commonly used, traditional synthetic meshes often face limitations such as poor integration with host tissue and complications like infection or adhesion formation. This paper presents hydrogel-based materials as a novel approach to addressing these abdominal wall defect repair challenges. Hydrogels, characterized by their biocompatibility, tunable mechanical properties, and ability to support tissue regeneration, offer a promising alternative to existing methods. This paper explores the design and application of these hydrogels, highlighting their potential to improve surgical outcomes through enhanced tissue integration, reduced inflammation, and minimized postoperative complications. Preclinical and clinical evidence suggests that hydrogel technology could revolutionize the standard of care for abdominal wall defects, offering a more effective and patient-friendly solution.

Keywords: Hydrogel; Abdominal wall defects; hernia; electrospinning; nanotechnology; tissue regeneration

Introduction

Abdominal wall defects (AWDs), a congenital disability, occur when the digestive organs such as the stomach or intestines- protrude from the body through an abnormal opening in the abdomen. The abdominal wall plays a crucial role in fetal development, as it should enclose these organs as they move into the abdomen. An abdominal wall defect occurs when this process is disrupted, allowing some organs to remain outside the abdomen [1]. The abdominal wall is undeniably a crucial weight-bearing structure in the body, playing a pivotal role in supporting respiratory mechanics and providing an indispensable protective barrier for internal organs. It dynamically adjusts to intra-abdominal and external pressures, constituting an indispensable component for normal physiological functions. Abdominal wall defects predominantly involve the absence of one or more components of the abdominal wall, and it is essential to note that postoperative incisional hernias are the prevailing type, representing over 65% of all abdominal wall defects [2,3]. These defects can arise from congenital anomalies, trauma, surgical procedures, or infections, and they present significant challenges in clinical practice due to their impact on patient quality of life and the complexity of repair strategies [4-5]. The two most common types of AWDs are gastroschisis and omphalocele, each with distinct characteristics and associated clinical challenges. They are of significant concern in neonatology and pediatric surgery due to the complexity of management and the long-term outcomes associated with these conditions [6,7].

Gastroschisis

Gastroschisis is an abdominal wall defect on the right side of the umbilicus. The absence of a protective covering over the protruding abdominal contents characterizes it. The exact cause is not fully understood, but it is believed to result from an abnormality in the lateral ventral body folds migration during early embryonic development, leading to a defect near the midline. Usually, the developing intestine moves outside the abdominal cavity around the sixth week of gestation. Over the next four weeks, the intestine undergoes a process of midgut rotation and returns to the abdomen. However, if the abdominal wall fails to form correctly, the intestine may remain outside the abdomen in the amniotic cavity [8,9].

Omphalocele

Compared to gastroschisis, which is characterized by a defect in the abdominal wall, omphalocele manifests as a herniation at the abdominal midline, explicitly involving the umbilical ring. This results in forming a 3-layer sac that encases the herniated abdominal contents. The layers of this sac consist of an inner layer of peritoneum, a middle layer of Wharton's jelly, and an outer layer of amnion. From an embryological standpoint, omphalocele is postulated to arise from a folding defect occurring during the bowel's return to the abdominal cavity during normal development [7].

While both conditions occur in approximately 4% of live births, omphalocele, which is detected during the second trimester of pregnancy, can have an incidence of up to 1 in 1,100. It is important to note that omphalocele is often accompanied by a high rate of intrauterine foetal death [8-10]. However, gastroschisis has become more common globally in the last several decades. In addition to gastroschisis and omphalocele, other less common abdominal wall defects include umbilical hernias and bladder exstrophy. Each of these conditions results from the failure of the abdominal wall to close properly during fetal development, leading to varying degrees of herniation of abdominal contents [11]. A large number of people around the world are affected by abdominal wall abnormalities, which are becoming more common as a clinical condition. Between 9% and 20% of the global population suffers from this illness. Healthcare expenditures exceed 10 billion USD annually due to over 400,000 reconstructive surgeries to correct internal soft tissue anomalies [12,13].

In the 19th and 20th centuries, medical science began to approach these defects with a more systematic and scientific methodology. Early surgical interventions were attempted, but these were often crude and fraught with high mortality rates due to the lack of anaesthesia, asepsis, and an understanding of the pathophysiology of these defects [6,7,14]. The development of pediatric surgery as a specialized field in the mid-20th century marked a turning point in managing abdominal wall defects. Surgeons like William Ladd [6] and Robert Gross [15], pioneers in pediatric surgery, contributed significantly to advancing surgical techniques and postoperative care, greatly improving survival rates.

At present, several options for the management of abdominal wall defects include tension-free mesh repair [16], abdominal wall tissue separation [17], flap reconstruction [18], abdominal wall expansion techniques [19], and temporary abdominal closure measures [20, 21]. However, these technologies can partially reconstruct, restore, or even compensate for the lost function while revealing the essential vulnerabilities. They include infection and immune rejection, high recurrence rate, and potential risks of reoperation, which set limits to reconstructing the advanced functions for humans and limit their clinical application [22-24].

To address the limitations of current treatments, researchers have explored the development of advanced biomaterials that promote tissue regeneration and integration with host tissues [25]. Tissue engineering is gradually becoming an innovative technique in managing abdominal wall defects (AWDs), delivering novel solutions for restoring the abdominal wall. Conventional repair methods involve using synthetic meshes or harvesting autogenous tissues, which bear several problems, such as infection, rejection and poor integration with the surrounding tissue. Tissue engineering aims to overcome these drawbacks by creating new constructs that will mimic the native tissue of the abdominal wall [26,27]. These new techniques currently in use depend on the carrying capacity of scaffolds to translocate cells and for the delivery of bioactive molecules. This dynamic approach increases tissue healing and regeneration, thus creating a stage for further development of tissue engineering [28,29-31].

Another promising avenue in tissue engineering is the incorporation of bioactive molecules, such as growth factors, into the scaffold material. These molecules can be released in a controlled manner to enhance cellular processes critical for tissue repair, including angiogenesis, cell proliferation, and ECM production. Growth factors like vascular endothelial growth factor (VEGF) and essential fibroblast growth factor (bFGF) have been shown to accelerate the formation of new blood vessels and improve the overall healing response in tissue-engineered constructs, leading to better outcomes in abdominal wall repair [32]. Tissue engineering scaffolds are designed to offer mechanical support and determine favourable local conditions to promote cell attachment, mobility, proliferation, and differentiation [33]. Hence, efforts to develop and manufacture a novel bio scaffold for abdominal wall tissue engineering are an active research focus on rebuilding the mechanical and biological features of the abdominal wall tissue.

Hydrogels

Hydrogels are crosslinked polymeric structures that provide three-dimensional hydrophilic structures capable of holding a massive amount of water or biological fluids. Due to their hydrophilic property and flexible nature, they are highly similar to natural tissues, which is why they are widely employed in the biomedical field for drug delivery, wound healing and tissue engineering. Hydrogels have been a topic of interest in recent years because of their multi-faceted characteristics and their possible application in enhancing several therapeutic interventions [34,35]. Some of the tunable properties associated with hydrogels, irrespective of whether they are composed of natural polymers, synthetic polymers, or a combination of both, include the following: This is particularly important for applications that need to interface with living tissues since gelatin, alginate, and chitosan are all biocompatible and biodegradable natural polymers. Polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyacrylamide are few examples of synthetic polymers which are used for specific Biomedical applications due to their ability to control mechanical properties, swelling behavior, and degradation rates [36,37]. Hydrogels have a three-dimensional crosslinked polymer network and can swell in water without dissolving, converting into a gel-like substance. Crosslinking can be done by physical contact (hydrogen bonds, ionic interactions) or by chemical means (covalent bonds). Crosslinking density is one of the most significant factors controlling the mechanical properties, pore size, and swelling behaviour of hydrogels used for biomedical applications [34]. Hydrogels have been used in many novel applications to manage abdominal wall defects. The one that holds much promise is their application as a scaffold for cell delivery. Thus, when stem cells or other types of regenerative cells are incorporated into a hydrogel structure, it becomes possible to produce a scaffold that fosters tissue healing. These cellular and nutrient-containing hydrogels can be applied directly to the defect area, where they offer the physical environment and encourage the formation of new, healthier tissue [38,39]. Growth factors and cytokines involved in the repair process have also been incorporated into hydrogels. For example, once introduced into hydrogels, angiogenic factors like vascular endothelial growth factors (VEGF) improve blood vessel formation, which is essential for supporting tissue nutrition under regeneration. Likewise, the hydrogels containing anti-inflammatories could prevent the immune response that leads to fibrosis and scarring that could hinder the repair [40].

Hydrogels have been extensively utilized in abdominal wall defects, mainly in regeneration, which involves providing scaffolds to hold tissues. This is because hydrogels can be designed to stimulate cell attachment, growth, and development into functional tissue. The hydrogel further enables this regenerative capacity to help retain moisture known to support healing and deliver bioactive molecules like growth factors at the site of the wound [41,42]. For example, when injury damages the abdominal wall extensively, the body may not be capable of growing enough new tissue to cover the gap; hydrogels can be used to provide a framework for tissue regeneration until the affected area is once again intact. These scaffolds can be designed to self-diminish in a time scale matching tissue regeneration, thereby minimizing the need for follow-up surgeries to remove the implant [43]. Hydrogels are also being considered drug delivery vehicles in the repair of the abdominal wall. They can be pre-dosed with antibiotics or anti-inflammatory drugs or with another therapeutic agent programmed to be released gradually to control the infection or inflammation at the surgical site. This ability to provide a slow

release is exceptional and is mainly used in preventing infections after surgery or adhesion formation in operations involving the abdominal wall [44]. For example, a hydrogel containing anti-microbial agents may be applied after surgery to minimize bacterial growth on the repair site, hence minimizing infection chance, which is a considerable issue with synthetic meshes [45].

Nanotechnology

Nanotechnology has brought significant advancements in developing new reparative techniques for abdominal wall defects and utilizing the subsequent innovative scaffolds and biological meshes widely applied in hernia repairs. Substantial progress has been made in developing synthetic and biological meshes in this critical area [26,46]. The traditional use of synthetic meshes in repairing these defects has been associated with complications such as infection, adhesion, inflammation, and poor integration with surrounding tissues. The application of nanotechnology in treating abdominal wall defects has emerged as a promising approach to address these issues. Nanotechnology involves manipulating materials at the nanoscale to enhance their physical, chemical, and biological properties, thereby offering improved outcomes in surgical repair [47].

The marked study has shown that myoblast-seeded bovine tunica vaginalis can be a scaffold to reconstruct major and extensive abdominal wall defects and regenerate skeletal muscle tissue [48]. In another study by Song et al., a modified plasma polymerization method generated A composite scaffold with acellular dermal matrices (ADM) and VEGF-loaded multi-walled carbon nanotubes (MWNTs). Controlled release of VEGF by the plasma polymerization treatment offers a method to accelerate early revascularization, and the 3% MWNT composite scaffold was able to repair abdominal wall defects [49].

Although synthetic meshes have been studied and proven helpful in certain studies, Synthetic meshes are commonly used for abdominal wall reconstruction, specifically for support of weakened or damaged tissue. These meshes can be fabricated from polypropylene, polyester, or polytetrafluoroethylene (PTFE). They aim to be biocompatible, compliant, and robust so that the surgical mesh can be integrated into the body tissues without causing infection or even sometimes rejecting the mesh by the body [50,51]. Hernandez-Gascon and their colleagues have extensively researched computational modelling of hernia-repaired abdominal walls. Their use of finite element (FE) models of the abdomen with three synthetic meshes has revealed that surgical repair fails to fully restore normal physiological conditions in the abdominal wall [52]. On the other hand, biological meshes, including meshes made from human or animal-derived tissues, can attract blood vessels and regenerate through the infiltration of native fibroblast cells; they are superior to synthetic meshes [49].

In addition, electrospinning has become another well-liked approach in tissue engineering for creating fibrous membranes with a large surface-area-to-volume ratio and fibers of the nano-micro range [53,54]. Owing to its diversity in composition, electrospun fibrous membranes have been investigated in various tissue engineering fields, such as cardiac patches [55], wound dresses [56], and drug-delivering carriers [53]. The ideal mechanical strength of the fibres is also one of the significant advantages of this technique, which made it an ideal mesh option for abdominal wall hernia reconstruction in the past decade [57,58]. However, the row material for electrospinning is less receptive, and the final fibres developed are primarily hydrophobic, which may hinder their histological integration in hernia repair. To this end, the researchers have incorporated 3D-printed scaffolds in managing abdominal wall hernia reconstruction [59,60]. Thus, 3D printing, based on its flexibility and variability of changes in the structure and a large number of printing sources, can be considered a promising trend for creating complex hernia repair materials in the future [61]. This potential makes us optimistic about the future of hernia repair with enhanced biocompatibility and mechanical strength. It is essential to mention that in recent years, much attention has been paid to hydrogels, electrospun fibrous membranes, and three-dimensional scaffoldings, as shown in Figure 1 [25].

This review will identify the advantages of hydrogel and nanotechnology and their use in reconstructing and remodelling defective abdominal walls. It will expound on new developments appearing in this branch of study; they give information about how they improve the design,

development, and challenges in hydrogel and nanotechnology-based repairs for abdominal wall defects, emphasising their application in regenerative medicine and surgery.

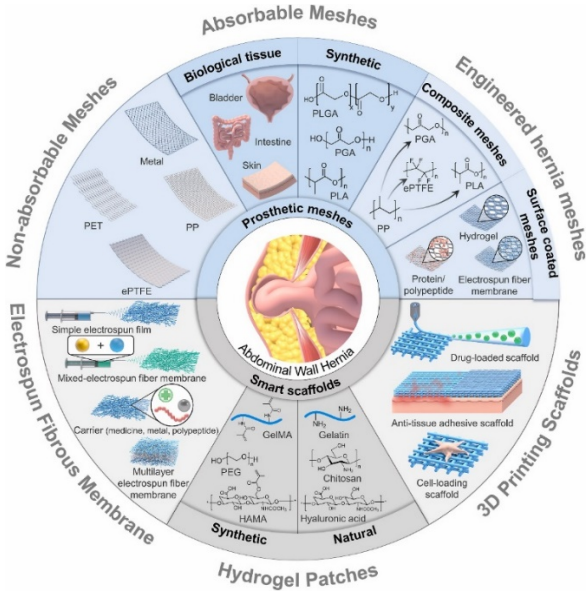

Figure 1. Schematic illustration of abdominal wall hernia repair meshes: from prosthetic meshes to smart materials [25].

Abdominal wall anatomy

The abdominal wall structure involves the skin, subcutaneous tissue, fascia, muscle, and peritoneum layers. The muscles are in layers; the big one is the rectus abdominis in the middle and the external, internal and transversus abdominis muscles on the sides. The muscles are also anchored by the fascia, especially the transversalis fascia, which offers more support. However, the anterior abdominal wall has the primary function of shielding the organs and ensuring appropriate positioning of abdominal organs as well as maintaining integrated intraabdominal pressure but at the same time is accountable for the majority of the abdominal wall injuries [62-64]. It is important to emphasize that hernias and other kinds of injury of the abdominal wall are not rare and can be caused by numerous factors, such as congenital abnormalities, trauma, surgery, and chronic diseases. They include hernias, which are a condition that results from the protrusion of abdominal contents through a hole in the abdominal muscles and traumatic injuries that lead to the tearing or injuring of the abdominal muscles [65-67].

To meet the needs of diagnosis and treatment, depending on the severity of abdominal wall defects, they can be divided into three different types: (a) Partial thickness defect of the abdominal wall is thus defined as those situations where only the skin and a part of the subcutaneous tissue is lost. These injuries may be due to trauma, surgical excisions, or ulcers and generally affect only the skin and subcutaneous tissue in the abdominal wall up to the dermal layer and occasionally the subcutaneous fat. These defects typically do not penetrate the deeper layer of the fascia or muscular layer. Consequently, they are usually not as extensive as they necessitate more complicated surgeries, such as free flap reconstruction, but only moderate surgery that may include skin grafting or local flap [68-72]. (b) Muscle and fascia atrophy or aplasia is another condition defined by the fact that the abdominal wall muscles and fascial tissues are missing while the skin remains. For instance, this condition might be congenital in prune belly syndrome, where the abdominal muscles are poor or absent. Skin, though, has lost deeper investing musculature and cutaneous fascia, and the integrity of skin tissue continues to be unperturbed, which creates certain biomechanical enigmas for the abdominal wall [73,74]. (c) Abdominal wall agenesis or abdominal wall aplasia is a condition where the abdominal wall is not formed at all (agenesis and aplasia means that an organ, tissue or body part failed to develop naturally; in some cases, it means that it is missing) [75-78]. Abdominal wall defect patients often have symptoms and signs of the primary disease and possible complications like adhesion and intestinal obstruction. Such complications stem from multiple abdominal injuries and infections [79-81]. The method of

separating the adhesions may cause damage to the adjacent intestinal wall tissues and eventually lead to the formation of intestinal fistulas [82,83]. For instance, patients suffering from an open abdomen often undergo numerous abdominal operations and recurrent abdominal lavage that continuously injures the abdominal wall. For instance, prolonged bed rest, prolonged abdominal surgery, and limited mobility lead to the atrophy and loss of contractile strength of the abdominal wall muscles, making it challenging to reconstruct the abdominal wall [84,85] effectively. This means definitive abdominal wall repair and reconstruction should be performed immediately.

Hydrogel-based abdominal wall repair

Thus, there has been an increasing focus on tissue-engineered scaffolds for better biocompatibility and functionality in abdominal wall repair. Among these, hydrogels have renewed interest in their application due to their versatile properties. Hydrogels are relatively more biocompatible and can be synthesized to disintegrate over time as the new tissue grows, thereby reducing the chances of post-surgery complications [86,87]. Different kinds of hydrogels have been investigated for AW repair, and each has unique characteristics that make it appropriate to be used in specific situations [87,88]. Bio-natural hydrogels like collagen-based, gelatin, or hyaluronic acid hydrogels have better biocompatibility and are degradable in the body. These materials are most suitable in applications where close interaction with the tissues, as well as the regeneration of tissues, is required. However, in many cases, their mechanical strength is insufficient to support significant or highly stressed defects [41,89-91]. Polyethylene glycol, polycaprolactone, and polyvinyl alcohol-based synthetic hydrogels can be developed to achieve better mechanical properties and tunable degradation rates. These materials can then be tailored to provide mechanical strength in the range needed for abdominal wall reconstruction while being biocompatible.

Moreover, synthetic hydrogels, with relatively greater mechanical strength, can also be surface-modified with bioactive agents to augment the therapeutic potential of the scaffolds [92-94]. Moreover, cross-linking is the critical parameter that defines the hydrogel's characteristics and potential fields of usage, and various approaches to cross-linking result in hydrogels with various physicochemical properties and network morphologies. The cross-linking of hydrogels may be categorized into two, namely, the physical cross-linking and the chemical cross-linking [95,96]. Physical cross-linking is formed through molecular interactions, including ion, hydroxyl and hydrogen bonds and crystallization was shown. Hydrogels prepared by this approach are generally stimuli-responsive and have good biocompatibility and degradability features. When hydrogels are applied to treat the defects of the abdominal wall, the biocompatibility of these materials is very low, significantly reducing the risk of inflammatory reactions [97]. The hydrogel's mechanical strength, porosity and degradation rate can be altered by controlling the parameters such as the polymer concentration, cross-linking mechanism and degree of cross-linking so that it mimics the defect site. This way, they can offer appropriate mechanical support and slowly dissolve as the repair process progresses and new tissue forms [98]. In light of this approach, the hydrogel can play a dual role of providing support to the tissue at the most necessary time during the healing process and then gradually disintegrating to let the tissue naturally regenerate, thus improving the repair process. Synthetic and natural hydrogels have attracted much attention for repairing abdominal wall defects because of the characteristic functional properties that can be designed for reconstructive surgery. Some of these properties are biocompatibility, high mechanical strength, controllable rates of degradation, and the possibility of incorporating bioactive molecules [99-101].

The following discussion provides an overview of the fundamental functional properties of hydrogels that make them suitable for repairing abdominal wall defects.

Biocompatibility

Biocompatibility is a critical property of any material used in tissue repair, and hydrogels excel in this regard. This property allows them to be safely employed in various medical applications, such as abdominal wall repair. Hydrogels' biocompatibility can be further divided into blood compatibility and

tissue compatibility. Due to their direct contact with blood, the assessment of blood compatibility is an important criterion for the successful application of hydrogels in abdominal wall repair [100-102].

Mechanical strength

The mechanical properties of hydrogels are also relevant when it comes to their application in the repair of the abdominal wall since the material is subjected to continuous mechanical stress. As noted before, the stomach wall withstands various mechanical forces. Thus, the implant used to repair the abdominal wall needs to offer support in preventing hernia relapse and allow the muscles of the stomach wall to move with ease. Depending on the concentration of the polymer, the mode of cross-linking and the extent of cross-linking, hydrogels can be designed to have mechanical properties of the target tissue. This tunability makes it possible for hydrogels to be used as scaffolds to support tissues during the initial stages of healing. In contrast, the steady degradation of the hydrogel scaffold enables replacement by the newly formed tissue, which in most cases takes the entire load [25,102,103].

Tunable degradation rates

The best and most desirable characteristic of hydrogels is that these gels can be made biodegradable and degrade in a controlled manner. It is thus possible to control the degradation rate of a hydrogel via the alteration of the chemical characteristics of the polymer matrix and the cross-linking density. Specifically, for the application of abdominal wall repair, the hydrogel must degrade in a way compatible with the tissue healing process. Hydrogel with rapid degradation may not provide adequate support in the initial healing period; on the other hand, hydrogel with a slow degradation rate may hinder tissue formation or cause complications such as inflammation or fibrosis. Since the degradation rate is adjustable, hydrogels can also stabilize the wall of the abdomen for a certain period before it makes room and remodels with healthy new tissue [104,105]. A study on real-time monitoring the material degradation and tissue remodelling utilized near-infrared fluorescent dye Cy5.5 NHS ester for labelling ECM composites (ECMB) performed from small intestinal submucosa (SIS) and CS/elastin electrostatically spun nanofibers. After implantation, the Cy5.5 ECMB composite material showed substantial fluorescence and had a degradation period of 16 weeks before the total breakdown occurred. The Cy5.5 ECMB composite implant thickness substantially grew at 4, 8, and 16 weeks after implantation, reaching a thickness similar to the standard abdominal wall at 16 weeks. Furthermore, the decrease in fluorescence was significantly and positively correlated with the thickness of the implanted Cy5.5 ECMB composite implant ($r = 0.9832$), suggesting a strong link between the degradation of the ECMB composite and tissue remodelling. Therefore, it not only achieved real-time monitoring of material degradation but also employed the thickness of the implant as an intuitive indicator for assessing the efficiency of tissue remodelling in ECMB composites, which provides new insights for evaluating and monitoring the tissue repair effectiveness of hydrogel [102].

Porosity and permeability

The most relevant aspect in the abdominal wall repair case is the hydrogel's porosity. Hydrogels are hydrophilic networks with interconnected pores that enable the permeation of nutrients and oxygen needed for cell growth and tissue repair. The size of the pores and their distribution in the hydrogel can be regulated during fabrication to enhance the ability of the cells to penetrate the structure and promote the formation of vessels, which is also essential for tissue integration. Furthermore, depending on the type and application of hydrogels, their permeability can also be altered and designed to release specific therapeutic agents, such as growth factors or antibiotics, to improve the injured part's healing process and prevent infection [98,106-108].

Bioactive molecule delivery

Hydrogels as suitable carriers for bioactive molecules can pave the way for enhanced tissue regeneration in abdominal wall repair. These bioactive molecules can be encapsulated into the hydrogel network and delivered at the site of tissue repair, and in this way, stimulate tissue regeneration. This property makes it suitable when used in abdominal wall repair because the area is sensitive to infections

due to the presence of the gastrointestinal system. Incorporating various antimicrobial agents directly into hydrogels is possible to prevent postoperative infections. Furthermore, hydrogels can incorporate growth factors that encourage further tissue reconstruction or anti-inflammatory drugs that reduce inflammation, thus preventing inflammation-induced chronic diseases. Thus, notwithstanding the synthetic nature of certain hydrogels, they have an advantage over synthetic meshes in releasing these therapeutic agents in a localized and sustained manner [109,110].

Tissue adhesion prevention

Another functional characteristic of hydrogels is that they help avoid adhesion formation between the abdominal wall and other tissues. Surgical adhesions occur frequently after abdominal operations and are associated with chronic pain, bowel obstruction and other complications. Designing hydrogels with anti-adhesive properties can be achieved by adding molecular agents, which reduce the ability of tissues to adhere to one another. For instance, hyaluronic acid-based hydrogels have demonstrated a decrease in adhesion formation because of their inherent property of lubrication and their ability to form a barrier to the tissues [25,41,87,111].

Hydrogels to repair the abdominal wall defect

Hydrogel has been widely applied for repairing abdominal wall defects because it has six unique characteristics that have contributed to this area's development. Yin et al. synthesized carboxymethyl chitosan and 4-arm poly (ethylene glycol) aldehyde for bio-multifunctional composite hydrogels for full-thickness abdominal wall defect. The histomorphological analysis reveals that as compared to the clinically used compact polypropylene mesh, the developed hydrogel patches promote the augmentation of enhanced thickness and integrity of the abdominal wall tissue by stimulating Ki67 expression, promoting the synthesis of collagen, promoting neovascularization, and reducing inflammation by down-regulating the levels of IL-6, TNF- α and IL -1β. The outcomes indicated that a bio-multifunctional hydrogel patch is feasible to be used on full-thickness abdominal wall defect treatment [112]. Another study was done by Wang et al. [113], where they developed a new biomaterial which is small intestinal submucosa coated with gelatin hydrogel containing essential fibroblast growth factor. They assessed the new biomaterials for use in abdominal wall reconstruction. It has been concluded from the results that the small intestinal submucosa coated with gelatin hydrogel incorporating basic fibroblast growth factor would be more effective in the regeneration and remodelling of host tissue to reconstruct the abdominal wall defects. Hu et al. fabricated a dopamine-modified hyaluronic acid and gelatin hydrogel with an acylation process. They further improved the hydrogel's antibacterial properties by incorporating silver nanoparticles [114]. Moreover, drugs can be loaded in hydrogels using their swelling characteristic, one in which the hydrogels are soaked in the drug-containing fluids. Moreover, drugs can also be impregnated in hydrogels by using the swelling character of the hydrogels through the use of liquid containing the drug.

Liu et al. synthesized a double-layer structured nanofiber membrane (GO-PCL/CS-PCL) of polycaprolactone (PCL), chitosan (CS) and graphene oxide (GO) through the process of continuous electrospinning. For enhancing the bio-functions (angiogenesis/reduction of ROS) of the patch (GO-PCL/NAC-CS-PCL), N-acetylcysteine here loaded was used to repair full-thickness abdominal wall defects (2 × 1. 5cm) in rat model. The results showed that double-layered nanomembranes described in this paper have notable anti-hernia and anti-adhesion capacities, and the in vivo micro-environment was significantly enhanced. It is thus applicable for repairing abdominal wall defects and has good features as a post-operative anti-adhesion key [115]. Dong et al. used the biodegradable polymer poly(lactic acid) PLA and poly(*N-isopropyl acrylamide*)-bpoly(ethyleneglycol) (PNIPAAm-b-PEG) to develop thermoresponsive hydrogel scaffolds that employed electrospinning technique. The composite electrospun scaffolds were seeded with rat adipose-derived stem cells (ADSCs). Scanning electron microscopy (SEM) assay for PNIPAAm-b-PEG/PLA scaffolds showed that its surface was covered with more adsorbed, well-spread, and stretched ADSCs in polygonal form compared to PLA and polypropylene (PP) scaffolds. It also showed that PNIPAAm-b-PEG/PLA mimetic hydrogel scaffolds

enhanced the surface for cell adhesion. These outcomes predict the ability to boost cell adherence and growth rates by employing hydrogels with proficient cell loading capacity [116].

Hydrogels have proved to be preferred by surgeons because they are easy to manipulate, can be injected, stick to the tissue surfaces and offer mechanical support. These hydrogels can be formulated to have controlled viscosity and cohesiveness so that surgeons can easily apply or mould them. These hydrogels can be designed in injectable systems so they may be accurately placed in the defects of the abdominal wall by using endoscopic approaches with syringes or catheters [117]. Deng et al. synthesized a hydrogel with in situ cross-linked CS-HA via Schiff base reaction, which can be injected in situ to fill the defect site in the abdominal wall [118]. Moreover, hydrogels can possess attributes like shear thinning or self-healing abilities, depending on the design. To be used as injectable or diffusible materials into the tissue, shear-thinning hydrogels demonstrate a decrease in viscosity under shear stress [119]. Once the shear force no longer exists, the hydrogels regain their original viscosity and offer stability and support to the repaired tissue after abdominal wall defect closure. Hydrogels' self-repairing ability depends on the change of time and the dynamic interactions within the functional groups within them. This means that the hydrogels can change their structure without any external stimulus, improving the handling of the gels following mechanical injury. For instance, applying hydrogel that exhibits self-healing capabilities in a sutured postoperative incision helps avoid patch failure or cracking [112].

Surgical meshes from synthetic and biological materials are standard for repairing abdominal wall defects. However, existing meshes still need to fully meet clinical requirements due to issues with biodegradability, mechanical strength, and tissue-adhesive properties. Nishiguchi et al. developed biodegradable, decellularized extracellular matrix (dECM) patches reinforced with a water-insoluble supramolecular gelator to address these issues. The physical cross-linking networks formed by the gelator improved the mechanical strength of the dECM patches, making them more effective in treating abdominal wall defects. These reinforced patches showed higher tissue adhesion strength and stability than the original dECM. The reinforced dECM patches promoted collagen deposition and blood vessel formation during material degradation in animal studies using a rat model with abdominal wall defects. Significantly, they also suppressed the accumulation of macrophages compared to nonbiodegradable synthetic meshes. These results indicate that the reinforced dECM patches have great potential for repairing abdominal wall defects due to their tissue-adhesive and biodegradable properties and improved mechanical strength enabled by the supramolecular gelator [120]. Implantable meshes are revolutionizing tension-free repair operations for internal soft-tissue defects. Liang et al. have pioneered a groundbreaking biocompatible Janus porous poly (vinyl alcohol) hydrogel (JPVA hydrogel) for optimal internal soft-tissue defect repair. This innovative JPVA hydrogel patch is designed to combat visceral adhesion, promote defect healing, and resist deformation, making it a promising solution for addressing internal soft-tissue defects. Research has demonstrated the JPVA hydrogel's remarkable success in facilitating abdominal wall repair and driving substantial defect regeneration in experimental models [121]. The tissue engineering process includes methods of 3D bioprinting [122], electrospinning [123], microfluidics [124], and self-assembly [125] for the development of hydrogels with highly organized structures that would replicate the environment of abdominal wall tissues. These methods enable cells and bioactive substances to be incorporated into hydrogels in specific locations, thus improving hydrogel's potential for reparative tissue regeneration and managing defects in the abdominal wall. Also, recent advances in this area mean the work has progressed further with hydrogel applications. Nevertheless, further investigation is still required to evaluate the biocompatibility, efficacy and tensile strength of hydrogel indicators for treating abdominal wall defects and regeneration.

Conclusions

The treatment of abdominal wall defects remains a significant challenge in surgical medicine, with conventional repair techniques often falling short due to limitations such as poor tissue integration, risk of infection, and postoperative complications like adhesion formation. The introduction of hydrogel-

based materials as a novel solution has sparked considerable interest in the field, offering a promising alternative that addresses many of these shortcomings. This discussion delves into the conclusions drawn from current research on hydrogel-based solutions for abdominal wall defects and explores the prospects of this innovative approach in clinical practice. Hydrogels have emerged as a versatile and practical material for repairing abdominal wall defects due to their unique properties, including biocompatibility, tunable mechanical strength, and the ability to promote tissue regeneration. Unlike traditional synthetic meshes, which can elicit foreign body reactions and fail to integrate seamlessly with host tissues, hydrogels can be designed to closely mimic the extracellular matrix (ECM) closely, facilitating better interaction with surrounding tissues. One of the critical advantages of hydrogels is their ability to be engineered with specific mechanical properties that can be tailored to match the dynamic environment of the abdominal wall. This adaptability is crucial, as the abdominal wall undergoes constant movement and pressure changes, necessitating a repair material that can flex and adapt without compromising structural integrity. Studies have shown that hydrogels can be formulated to provide the necessary mechanical support while remaining sufficiently flexible, thus reducing the risk of complications associated with rigid synthetic materials.

Moreover, hydrogels can be loaded with bioactive agents such as growth factors, cytokines, or antimicrobial peptides, which can further enhance tissue regeneration and reduce the risk of infection. This multifunctionality allows hydrogels to serve as a physical barrier and an active participant in the healing process, promoting faster and more effective recovery. For example, hydrogels incorporating vascular endothelial growth factor (VEGF) have significantly enhanced angiogenesis, which is vital for tissue repair and regeneration. In addition to promoting tissue integration and healing, hydrogels offer a more favourable biocompatibility profile than traditional materials. The risk of chronic inflammation, a common issue with synthetic meshes, is significantly reduced with hydrogels, as they are designed to degrade over time into non-toxic byproducts easily resorbed by the body. This controlled degradation aligns with the natural healing process, allowing the hydrogel to provide support during the critical early stages of healing and gradually transfer the load to the newly formed tissue as it matures. Clinical studies have provided encouraging results, demonstrating that hydrogel-based repairs can lead to fewer complications and better overall outcomes than traditional methods. Patients treated with hydrogel materials have shown reduced infection rates, lower incidence of adhesion formation, and improved functional outcomes, such as greater flexibility and reduced pain during recovery. These findings suggest that hydrogel-based solutions could become the new standard in the surgical repair of abdominal wall defects.

Future Perspective

While the current research on hydrogel-based solutions for abdominal wall defects is promising, several areas require further investigation before these materials can be widely adopted in clinical practice. One of the primary challenges is the need for long-term studies to assess the durability and safety of hydrogel implants over extended periods. Although short-term results are favourable, it is essential to understand how these materials perform over the years, particularly in terms of their mechanical stability, biodegradation rates, and potential for late-onset complications. Future research should also focus on optimizing the composition of hydrogels to match the specific requirements of different types of abdominal wall defects. For example, hydrogels' mechanical properties and degradation rates may need to be adjusted depending on whether they are used to repair a small hernia or a significant, complex defect.

Additionally, incorporating advanced bioactive agents within the hydrogel matrix could be further refined to enhance their therapeutic potential. The use of personalized medicine approaches, where hydrogels are tailored to the specific biological environment of each patient, represents a promising direction for future development. Another exciting prospect for hydrogel-based solutions is their potential application in minimally invasive surgery. As surgical techniques continue to evolve, there is a growing emphasis on reducing patient morbidity and recovery times through less invasive procedures. Hydrogels are well-suited for such applications due to their flexible and injectable nature.

Injectable hydrogels could be delivered via laparoscopic techniques, allowing for precise placement with minimal disruption to surrounding tissues. This approach could significantly reduce recovery times and improve patient comfort, making hydrogel-based repairs an attractive option in modern surgical practice.

Moreover, developing "smart" hydrogels that respond to environmental stimuli, such as pH, temperature, or the presence of specific enzymes, could revolutionize how abdominal wall defects are treated. These innovative materials could be designed to release therapeutic agents in response to specific triggers, providing targeted treatment only when needed. For example, a hydrogel could be engineered to release anti-inflammatory drugs in response to early signs of inflammation, preventing complications before they arise. Such innovations would not only improve the effectiveness of the treatment but also reduce the need for additional interventions, further enhancing patient outcomes. The commercialization and regulatory approval of hydrogel-based products for abdominal wall repair will also play a crucial role in their widespread adoption. Collaborations between researchers, clinicians, and industry partners will be essential to translate the promising findings from the laboratory into commercially viable products. Regulatory bodies must establish clear guidelines for approving hydrogel-based materials, ensuring they meet safety and efficacy standards. Successfully navigating these regulatory pathways will be critical in determining the future availability of hydrogel-based solutions in the clinical setting. While challenges remain to be addressed, particularly regarding long-term performance and regulatory approval, the prospects for hydrogel-based solutions are highly encouraging. As research continues to evolve and more clinical data becomes available, hydrogels will likely become an integral part of the surgical toolkit for repairing abdominal wall defects, leading to improved outcomes and better quality of life for patients.

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Authors contribution

All the authors have contributed equally.

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Research Article

GC-MS analysis, antimicrobial and antiulcer evaluations of ginger-infused virgin coconut oil

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Abstract

Medicinal herb-infused oil is utilized in folkloric medicine due to its efficacy in ameliorating various diseases of humans; even with this evidence, most herbs are still underutilized and poorly investigated. This prompted the investigation of ginger-infused virgin coconut oil's chemical composition and antimicrobial and antiulcer activities (GIVCO). The virgin coconut oil was produced using the natural fermentation method. Dried ginger and VCO (1:10) were infused for three days and then filtered to obtain ginger-infused virgin coconut oil. GC-MS was used to detect GIVCO chemical constituents. The agar well diffusion method was used for antimicrobial evaluation test organisms. The ethanol and indomethacin-induced ulcer models were used for antiulcer evaluation. The GC-MS analysis identified the presence of lauric acid methyl ester, Myristic acid, Palmitic Acid, Oleic acid, Capric acid, Stearic acid, Caryophyllene, Docosahexaenoic Acid methyl ester. At 100 mg/ml, inhibition zone diameter (IZD) ranged from 10-16 mm for various strains of bacteria and 10-21 mm for various strains of fungi. The effect of GIVCO on tested organisms compared favorably to that of standard drugs. The acute toxicity study of GIVCO is atoxic. The antiulcer activity demonstrated a dose-dependent effect; at 100 mg/ml, the GIVCO protected the intestine with a %UI of 80 and 63 for ethanol and indomethacin model against the standard omeprazole with 50% UI. The study demonstrated the potential of GIVCO as an alternative medicine against antimicrobial infections and the prevention of stomach ulcers.

Keywords: infused oil; ginger; phytochemicals; ulcer, microorganisms; virgin coconut oil

Introduction

The efficacy of medicinal plants, especially herbal preparation in the treatment of diseases, has been regarded as the best alternative to synthetic medicine for health care management. The exploitation of medicinal plants started in ancient times and is invaluable as a rich source of therapeutic agents for the prevention and management of diseases all over the globe. Plants being used as nutritional supplements and medicine are more likely to yield pharmacologically active compounds, which are crucial for maintaining a healthy body [1,2]. The chemical compounds present in plants with the potential to maintain good health are known as phytochemicals. These phytochemicals have been in use from time immemorial for the treatment of different health disorders and are considered relatively safer compared to conventional drugs [3]. With the increasing popularity of plants as a safe and cheaper alternative to conventional therapeutic agents, the exploitation of plants as a whole or in the form of drugs has many

prospects for ameliorating health challenges. Herbal-infused oil is an easy herbal preparation that captures the benefits of herbs for many uses. The infusion of oil with herbs can transform them into medicinal preparations. Various dried herbs and organic carrier oils (virgin coconut oil, Jojoba oil, and olive oil) are the best choices as they have a long shelf life. Herbal-infused oil has demonstrated significant activities against disease conditions, as reported [4,5]. This formed the research background to investigate the potential of GIVCO to alleviate the burden of resistance pathogenic organisms arising from different strains.

However, this study aimed to evaluate the antimicrobial and antiulcer effects of GIVCO by utilizing the beneficial health effects of ginger rhizome and carrier oil properties of virgin coconut oil as an extracting solvent. The infused oil from this study is expected to be a putative therapeutic agent.

Materials and Methods

Materials

Collection and preparation of plant materials

Zinger officinale rhizomes and fresh Cocos nucifera nut were sourced from Ogbete main market Enugu, Nigeria. The samples were authenticated in the Department of Pharmacognosy Enugu State University of Science and Technology, Agbani, Enugu State.

Test organisms

The microorganisms were selected based on the ubiquitous infection they cause in the human population. They comprise two Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumonia),* two Gram-negative *(Escherichia coli, Klebsiella pneumonia) bacteria,* and the fungi used were *Aspergillus niger, Aspergillus flavus, Epidermophyton floccosum* and *Candida albican* strains isolated from wound, ear infection, and high vaginal swap (HVS). These microorganisms were clinical isolates sourced from Adonai Research Laboratory and Biomedical Services, Nsukka, Enugu state. The tubes containing the bacterial isolates were incubated at 37 °C for 24 h, while the fungi isolates were incubated at 28 °C for 48 h. The inoculum was standardized by adjusting its turbidity to correspond with the 0.5 McFarland standard to have a comparable density equivalent to approximately 108 CFU/ml.

Experimental animal

Twenty (20) (male and female) Albino Wister rats with body weights ranging from 116 gm to 130 gm were used for the experiment. The rats were allowed to acclimate in the experimental lab for 7 days, giving them access to a standard pellet diet and water ad libitum. The food was withdrawn 12 h before the experiment; however, they were allowed free access to water.

Methods

Processing of Z. officinale powder

Fresh Z. officinale rhizomes were washed thoroughly to remove soil particles. They were sliced into tiny shapes to aid easy drying and allowed to dry under shade for 10 days. An electric blender pulverized the dried rhizomes to a fine, coarse powder. The powdered sample was stored in a cool, dry cupboard and is awaiting further processing.

Processing of Virgin coconut oil (VCO)

The extraction of coconut oil begins with carefully cracking a fresh C. nucifera nut to collect the mesocarp. This mesocarp is thoroughly washed and sliced into small, box-shaped pieces, facilitating a smoother grinding process. A mechanical blender is employed to pulverize these pieces into delicate flakes. Following this, the coconut milk is extracted from the chaff using Mushin cloth and placed in an airtight plastic bucket, left undisturbed for 24 h. This step is crucial as it separates three distinct layers: the cord, oil, and water. The cord forms the upper layer and is gradually and carefully scooped out. Next, the oil is extracted precisely to avoid mixing it with the water layer beneath. The oil sample is then stored in a dry container and is awaiting further processing. This extraction method, utilizing

fermentation, not only optimizes the quality of the oil but also ensures a sustainable approach to producing a valuable product.

Preparation of GIVCO

10 gm of finely powdered ginger was weighed out into a conical flask. 100 gm of virgin coconut oil was added to the conical flask and stirred thoroughly. The mixture was heated in a water bath at 60°C for 30 min and allowed to stand for 72 h at room temperature. Thereafter, it was filtered using a funnel and cotton wool-clogged funnel to extract the GIVCO. The GIVCO sample was stored in a dry container and is awaiting further processing.

GC-MS profiling of ginger infused VCO

The ginger-infused oil was analyzed using GCMS-QP2010 PLUS (SHIMADZU, JAPAN). The capillary column type was DB-IMS [30 m (length) X0.25µm (diameter) X0.25µm (film thickness)]. The carrier gas used was helium at a constant flow rate of 19.9 ml/min and an average velocity of 36.2 cm/s; the pressure was 56.2 KPa. The initial column temperature was set at 6°C 0 for 1 min and increased by 3 °C/min up to 180 °C and to the final temperature of 280 °C at the rate of 6 °C/min; volume injected was 1.0 µl at 250 °C in the splitless mode. Mass spectra were obtained by EI at 70 eV over the scan range 10-1000 m/z. The compounds were identified by comparison of their mass spectra with those of the NIST mass spectral library.

Acute toxicity study

The acute toxicity study was conducted following established protocols [6]. In the initial phase, GIVCO was administered at 10, 100, and 1000 mg/kg to three distinct groups of three mice each. Notably, after 24 h, no fatalities were recorded, indicating a positive initial safety profile. In the second phase, we advanced to higher doses of 1600, 2900, and 5000 mg/kg. During the 24 h observation period, we carefully tracked any signs of mortality and potential behavioral changes. Encouragingly, no deaths or signs of toxicity were observed, further supporting the safety and tolerability of GIVCO at these tested doses.

Ethanol-Induced Ulcer Model

The ethanol-induced ulcer model was adopted as previously reported [7]. The study involved dividing rats into four groups, with each group comprising five rats. Group A served as the negative control, receiving ethanol without any treatment. Group B acted as the positive control and was treated with omeprazole to evaluate its protective effects. Group C was administered a treatment of 50 mg/kg bodyweight of GIVCO, while Group D received 100 mg/kg bodyweight of GIVCO, allowing for a comparison of dosage effects. To ensure that the treatments could be evaluated effectively, Groups B, C, and D were treated one hour prior to the administration of ethanol. Following a one-hour period post-ethanol exposure, the rats were euthanized through cervical dislocation for humane considerations. Their stomachs were then carefully excised and opened along the greater curvature. This careful dissection allowed for thorough examination under a dissecting microscope to assess ulcer formation and quantify ulcer scores. Subsequent calculations involved determining the ulcer index (UI) and the percentage of ulcer inhibition, providing valuable insights into the protective effects of the treatments administered.

Indomethacin- induced ulcer model

The indomethacin-induced ulcer model, as reported previously, was adopted [8]. The study divided rats into four groups, each comprising five rats. Group A served as the negative control, receiving 30 mg/kg indomethacin without any treatment. Group B acted as the positive control and was treated with omeprazole to evaluate its protective effects. Group C was administered a treatment of 50 mg/kg body weight of GIVCO, while Group D received 100 mg/kg body weight of GIVCO, allowing for a comparison of dosage effects. Groups B, C, and D were treated one hour before ethanol administration to ensure that the treatments could be evaluated effectively. Following one-hour post-ethanol exposure,

the rats were euthanized through cervical dislocation for humane considerations. Their stomachs were then carefully excised and opened along the greater curvature. This careful dissection allowed for thorough examination under a dissecting microscope to assess ulcer formation and quantify ulcer scores. Subsequent calculations involved determining the ulcer index (UI) and the percentage of ulcer inhibition, providing valuable insights into the protective effects of the treatments administered.

Table 1. compounds detected by GC-MS analysis of GIVCO.

Name of compounds	Retention time (min)	Molecular formula	Molecular mass (g/mol)
Limonene	2.49	$C_{10}H_{16}$	136.2
Benzaldehyde	4.04	C_7H_6O	106.1
Eucalyptol	5.86	$C_{10}H_{18}O$	54.25
Cymene	8.51	$C_{10}H_{14}$	134.2
Caryophyllene	14.06	$C_{15}H_{24}$	204.4
Stearidonic acid methyl ester	14.63	$C_{19}H_{38}O_2$	298.5
Humulene	20.44	$C_{15}H_{24}$	204.4
Lauric acid	21.07	$C_{12}H_{24}O_2$	200.3
Docosahexaenoic acid methyl ester	22.46	$C_{22}H_{32}O_2$	328.5
2-Bromo-5-methoxytoluene	22.80	C_8H_9BrO	201.1
Geranyl geranyl alcohol	23.18	$C_{20}H_{34}O$	209.5
Docosapentenoic acid methyl ester	23.80	$C_{23}H_{36}O_2$	344.5
Myristic acid	24.02	$C_{14}H_{28}O_2$	228.4
3,5-diphenyl-4-methyl-3-penten-2-one	24.19	$C_{18}H_{18}O$	250.3
Methyl stearate	26.63	$C_{19}H_{38}O_2$	298.5
Caprylic acid	27.21	$C_8H_{16}O_2$	144.2
2,5-dimethyoxythiophenol	28.22	$C_8H_{10}O$	122.2
Palmitoleic acid	28.53	$C_{16}H_{30}O_2$	254.4
2,4,5-trimethoxybenzaldehyde	28.57	$C_{10}H_{12}O_4$	196.2
Elaidic acid	29.15	$C_{18}H_{34}O_2$	282.5
Capric acid	29.34	$C_6H_{12}O_2$	172.3
Eicosapentaenoic acid methyl ester	29.47	$C_{21}H_{32}O_2$	316.5
Eugenol	30.06	$C_{10}H_{12}O_2$	164.2
Lauric acid methyl ester	31.39	$C_{13}H_{26}O_2$	214.3
Palmitic acid	33.03	$C_{16}H_{32}O_2$	256.4
Isopropyl palmitate	34.65	$C_{19}H_{38}O_2$	298.5
Stearic acid	36.424	$C_{18}H_{36}O_2$	284.5
Oleic acid	36.645	$C_{18}H_{34}O_2$	282.5

Antimicrobial evaluation (agar well diffusion method)

The antimicrobial evaluation of the GIVCO was assayed using the agar well diffusion method. Sixteen Petri dishes were set up, and the plate lids labeled Sa1, Sa2, St1, St2, Ec1, Ec2, Kb1, Kb2, Ca1, Ca2, Ca3, Ca4, Ca5, Af, An and Ef which represents Staphylococcus aureus strain 1, Staphylococcus aureus strain 2, Streptococcus sp. strain, Streptococcus sp. strain 2, Escherichia coli strain 1, Escherichia coli strain 2, Klebsiella pneumoniae strain 1, Klebsiella pneumoniae strain 2 respectively. Ca1 and Ca2, Candida albican from the vaginal swab, Ca3 and Ca4, Candida albican from diabetic wound swab, Ca5 Candida albican from ear swab in otitis media, Aspergillus flavus Af, Aspergillus niger An, and Epidermophyton floccosum Ef respectively. Each plate was divided into sections with codes 1,2,3,4, and 5 representing the sample concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 10 mg/ml of standard drugs, respectively. The nutrient agar weighing 4.5 gm was dissolved in 160 ml of water in a conical flask. The SDA weighing 10 gm was dissolved in 160 ml of water in a conical flask, and 62.5 mg of chloramphenicol capsule was added to inhibit bacteria growth. The solutions were sterilized by heating in an autoclave for about 15 min at 120 °C and then cooled. 0.1 ml of the microorganisms was added carefully to the plates as labelled. 20 ml of molten agar was added to each plate containing the microorganisms with SDA for all fungi and nutrient agar for bacteria. It was appropriately swirled and allowed to solidify. The cork borer was used to make five holes of 8mm diameters on the sections labeled on the plate, and an additional hole was made in the middle for the control drug. The different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of GIVCO, virgin coconut oil, ginger powder solution, and 0.1 ml control drugs were added to the labeled bored holes using a micropipette.

The plates were allowed to stand undisturbed for a few minutes to allow diffusion. Then, they were stacked adequately in an incubator cabinet to prevent uneven heating and incubated for 24 h (37 °C) for bacteria and 48 h (28 °C) for fungi, respectively. At the end of the incubation period, the inhibition zonal diameter of microbial growth on the plates was observed, and the results were recorded in millimeters.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by multiple comparison tests. The values are expressed as means ± SEM and differences in the values were considered significant at 5 % level.

Results and Discussion

GC-MS Analysis

The GC-MS detected the presence of fatty acid esters as the major chemical constituents in GIVCO. The compounds identified, when compared with the NIST MS library, showed limonene, Benzaldehyde, lauric acid, Eucalyptol, Stearidonic acid methyl ester, caryophyllene, Cymene, Myristic acid, Docosahexaenoic acid methyl ester, Geranyl geranyl alcohol, Palmitoleic acid, Caprylic acid, Palmitic acid, Humulene, Lauric acid methyl ester, Methyl stearate, Eicosapentaenoic acid methyl ester, Omega-3-arachidonic acid methyl ester, and 2-phenyl-1-propanol as presented in Table 1. These novel findings are sure to pique the interest and stimulate further research in this area.

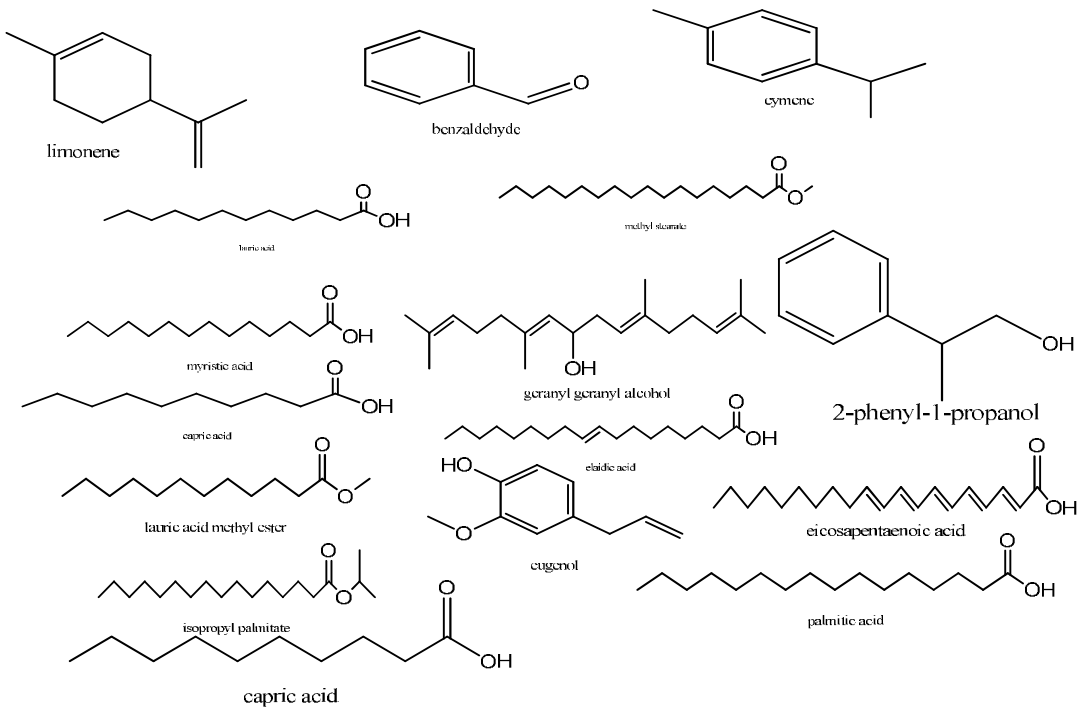

Figure 1. Structure of compounds identified in GIVCO.

Antimicrobial determination

The result showed that GIVCO has a significant effect against the two strains of S. aureus and S. typhi. One strain of E. coli whereas E. coli strain 2 and two strains of K. pneumoniae was not inhibited even at the high dose of 100 mg/ml, unlike the standard drug that showed potent inhibition even at a much lower dose, as presented in Table 2. In Table 3, the GIVCO exhibited a reasonable effect against the five C. albicans A. flavus strains. A potent inhibition was recorded against E. floccosum, whereas A. niger was not inhibited even at the high dose of 100 mg/ml. There is somewhat comparable effect between the standard drug and GIVCO.

Table 2. Effect of GIVCO on bacteria species.

Test samples	Conc. mg/ml	Sa1	Sa2	St1	St2	Ec1	Ec2	Kb1	Kb2
GIVCO	100	16	12	12	10	16	0	0	0
	50	14	10	10	10	14	0	0	0
	25	9	0	9	6	9	0	0	0
	12.5	0	0	0	0	0	0	0	0
Levofloxacin	0.01	36	36	36	36	36	36	36	36

Key: Sa1, Sa2, St1, St2, Ec1, Ec2, Kb1, Kb2, ca1, ca2, ca3, ca4, ca5, Af, An, Ef which represents Staphylococcus aureus strain 1, Staphylococcus aureus strain 2, Streptococcus sp. Strain 1, Streptococcus sp. strain 2, Escherichia coli strain 1, Escherichia coli strain 2, Klebsiella pneumoniae strain 1, Klebsiella pneumoniae strain 2.

Table 3. Effect of GIVCO on fungi species.

Test samples	Conc. mg/ml	Ca1	Ca2	Ca3	Ca4	Ca5	Af	An	Ef
GIVCO	100	21	20	14	10	16	13	0	28
	50	18	24	11	10	14	11	0	15
	25	12	15	9	6	9	9	0	10
	12.5	8	9	0	0	0	0	0	8
Fluconazole	0.01	45	45	45	45	45	45	45	45

Key: Ca1 and Ca2, Candida albican from vaginal swab, Ca3 and Ca4, Candida albican from diabetic wound swab, Ca5 Candida albican from ear swab in otitis media. Af, Aspergillus flavus, An, Aspergillus niger and Ef, Epidermophyton floccosum

Acute toxicity study

The acute toxicity study showed no mortality, even at 5000 mg/ kg within 24 h of administration of GIVCO. However, there was a visible sign of soft stool in the first phase, whereas in the second phase, there was a visiblesign of diarrhea.

Table 4. Initial acute oral toxicity test result.

Dose	10 mg/kg	100 mg/kg	1000 mg/kg
Surviving rat	3/3	3/3	3/3

Table 5. Final acute oral toxicity test result.

Dose	1600mg/kg	2900mg/kg	5000mg/kg
Surviving rat	1/1	1/1	1/1

Effect of GIVCO on ethanol induced ulcer in rat

The high dose of GIVCO exhibited the best antiulcer activity on the induced ulcer model with a percentage ulcer index of 80 %. There is a significant difference between the high dose of GIVCO and the standard drug, omeprazole. The low dose and standard drug showed no significant difference.

Table 6. Effect of GIVCO on ethanol induced ulcer.

Group	Treatment	Ulcer index	% ulcer index
Group A (Negative control)	Received 1mL water	10.7±0.2	-
Group B (Positive control)	30mg/kg Omeprazole + 1mL ethanol	5.36±0.2*a	50
Group C (Low dose)	50mg/kg GIVCO + 1mL ethanol	5.32±0.2a	50
Group D (High dose)	100mg/kg GIVCO +1mL ethanol	2.38±0.2*	80

Key: GIVCO= Ginger-infused virgin coconut oil, Values are expressed as Mean SD of each rat group (n=5). Mean values on the same column with (*) are significant at P < 0.01. While mean value with (a) are non-significant at P<0.01 and 0.05 respectively.

Effects of GIVCO on Indomethacin induced ulcer in rat

The effects of GIVCO on stomach ulcers caused by indomethacin demonstrated reasonable activity. The statistical analysis showed no significant difference between high and low doses of GIVCO, as indicated by a percentage ulcer index of 63 %. Comparing the standard ulcer drug omeprazole, there is a disparity in effect, indicating a significant difference.

Table 7. Effect of GIVCO on indomethacin induced ulcer.

Group	Treatment	Ulcer index	% ulcer index
Group A (Negative control)	Received 1mL water	10.7±0.2	-
Group B (Positive control)	30mg/kg Omeprazole + 30mg/kg indomethacin	5.36±0.2*	50
Group C (Low dose)	50mg/kg GIVCO +30mg/kg indomethacin	3.92±0.78*a	63
Group D (High dose)	100mg/kg GIVCO +30mg/kg indomethacin	3.94±0.78*a	63

Key: GIVCO= Ginger-infused virgin coconut oil, Values are expressed as Mean±SD of each rat group (n=5). Mean values on the same column with (*) are significant at P < 0.01. While mean value with (a) are non-significant at P<0.01 and 0.05 respectively.

Discussion

With the progress made in the use of synthetic drugs to combat the increasing rate of various ailments coupled with their adverse effects, much has not been done to alleviate the burden of health challenges, especially in developing countries. Therefore, the need for alternative therapeutic agents from indigenous herbs is crucial. GIVCO has demonstrated potential as an antimicrobial agent against different strains of bacteria and fungi. The antibacterial evaluation of GIVCO, at 100 mg/ml, showed a significant effect against Staphylococcus aureus 1 and 2 strain, Streptococcus sp. strains 1 and 2, and Escherichia coli strain 1 with IZD range of 10-16 mm Table 2. The result of the test sample was not comparable to the standard drug Levofloxacin. This could be because test samples could not diffuse well into the agar medium due to their compositions. For the fungi evaluation, the standard drug fluconazole showed the widest and clearest inhibition zone compared to other test samples. GIVCO had a significant antifungal effect on test organisms. At 100 mg/ml, GIVCO inhibited the growth of five different Candida albican strains, Aspergillus flavus, and Epidermophyton floccosum, with an IZD range of 10-28 mm, respectively Table 3. However, infused ginger oil showed more potent antifungal activity on Candida albican of different strains, Epidermophyton floccosum, and Aspergillus flavus. The potent inhibition recorded against Epidermophyton floccosum and candida albican strains indicated using GIVCO to treat skin and nail infections. The observed antimicrobial properties showed that virgin coconut oil could extract chemical constituents in ginger that are responsible for treating diseases associated with pathogenic organisms. Ginger has been reported to possess phytochemicals that inhibit microbial infections [9,10].

The acute toxicity study of GIVCO showed no mortality in the animals, though some physiological change was observed in Tables 4 and 5. At a concentration of 100 mg/kg body weight, the consistency of the droppings changes with 30 min of administration. The higher the dose, the rate at which the droppings get watering. This effect suggested the potential of GIVCO as a putative, purgative, and laxative agent.

A peptic ulcer is a localized lesion of the gastric or duodenal mucosa wall occurring at a site where the mucosal epithelium is exposed to aggressive factors, majorly chronic alcohol consumption, and misuse of non-steroidal anti-inflammatory drugs (NSAIDs). The ulcers induced by ethanol have been implicated in stimulating the formation of leukotriene C4 (LTC4), mast cell secretory products, and reactive oxygen species, resulting in the damage of gastric mucosa [11,12]. Moreover, NSAIDs, unlike ethanol, usually induce ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway [13,14]. The GIVCO demonstrated a reasonable effect on ethanol and NSAID-induced ulcers, as reported in Tables 6 and 7. The percentage ulcer index of GIVCO at 100 mg/kg outperforms the standard drug, suggesting a better antiulcer agent compared to omeprazole. The GIVCO ameliorates gastric ulcers caused by ethanol (80 % ulcer reduction) and indomethacin (63 % ulcer reduction) significantly at $p<0.01$, respectively.

The phytochemicals detected by GC-MS analysis of GIVCO, as presented in Table 1 and Figure 1, indicated the presence of phytochemicals of medicinal importance. The copious concentration of medium-chain fatty acid and its ester bioactive metabolites in GIVCO contributed to this study's observed antimicrobial and antiulcer properties. The fatty acids detected in the GIVCO, palmitic acid, myristic acid, oleic acid, and lauric acid, possess antimicrobial, antioxidant, and anti-inflammatory activities [15-18].

Conclusion

This study demonstrated that GIVCO contains phytochemical constituents, which contribute to the observed antiulcer and antimicrobial activity. Furthermore, this study has established the therapeutic potential of GIVCO in the treatment and management of diseases, thus recommending its utilization in the management of selected diseases.

Authors contribution

IJA designed the research work, JOO sourced and prepared the plant materials, and NHO drafted the first manuscript. CCA evaluated and interpreted the antimicrobial result. RCO produced the ginger-

infused virgin coconut oil, RMO assessed and analyzed the antiulcer result, and FBCO interpreted the GCMS chromatogram result.

Declaration of interest

The authors declare no conflict of interest.

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Review Article

PROTACs: Mechanism and Bioavailability enhancement strategies by nanotechnology, RNA viral infections (vaccine strategy) and Prodrug development

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Abstract

Proteolysis Targeting Chimera (PROTACs) are a brand-new concept of therapeutics that use the ubiquitin-proteasome system for selective degradation of disease-related proteins. Like other therapeutics, PROTACs function by recruiting a protein target and a ubiquitin ligase that leads to the degradation of the target protein; however, unlike most other drugs, PROTACs do not simply disable the target's function through steric hindrance. As a result of this present review, the application of PROTACs will be discussed in oncology, leukemia, and neurodegenerative diseases. However, there are considerable difficulties regarding the bioavailability of PROTACs; one of which is selecting the appropriate degron. This review describes the issues of bioavailability that are related with PROTACs, such as solubility and stability issues, and proposed tactical decisions, which could be used under those conditions. The newest approaches in nanotechnology-aided drug delivery and the use of nanocarriers to address the limitations in PROTACs and their pharmacokinetic and pharmacodynamic properties are discussed. They improve the solubility and stability of PROTACs, allow for targeted delivery to the tumour site and minimize toxicity to healthy cells. Moreover, arguably the most interesting virtue of PROTACs is its applicability for RNA viral infections (new vaccine design) based on the degradation of viral proteins. An idea for the field of antiviral therapy, this invention defines a new horizon for the management of diseases that have not responded to traditional methods. In addition, the review focuses on the advanced techniques on the application of prodrug strategies in PROTACs. These inactive PROTACs are then converted to prodrugs to enhance their uptake into target tissues thereby increasing the concentrations at the target site and at the same time minimizing on the adverse effects that may be caused by higher concentrations in the entire body. Also, when PROTACs are incorporated into sophisticated drug delivery systems, their desirability and selectivity are further improved beyond existing issues related to small-molecule inhibitors. This review also includes new progress, clinical applicability, and development trend of PROTAC for the change of disease therapy and drug delivery.

Keywords: PROTAC; targeted protein degradation; RNA viral infections; prodrug; Oncology; Alzheimer's disease; Parkinson's disease; nanocarriers

Introduction

Small molecule inhibitors and monoclonal antibodies are the two most used molecularly targeted types of drugs in the clinical treatments in today's global market. These drugs operate mechanism wise by counter looking for the active site of target proteins and functioning as competitive antagonist ligands thus restraining the proteins from binding to their subsequent targets. However, the binding sites can be altered through mutations of genes or the change in the conformation of the target protein leading to drug resistance. Most of the current research in target identification is therefore inclined to more conventional targets such as kinases and G protein coupled receptors. But this is slowly shifting

with technology crossing over to hard to 'drug' targets hence the name 'undruggable'. Approximately 80% of all proteins are targets and most of them include no enzymatic action [1].

PROTACs are a new generation of heterobifunctional small molecules that work on the basis of the UPS system [2]. These molecules consist of two ligands: one is to recognize the protein of interest (POI) and the other targets an E3 ubiquitin ligase. As the result, PROTACs effectively encourage the ubiquitination and, consequently, proteasomal destruction of the target protein by comingling the target protein and E3 ligase. This distinct outline of action gives several merits over conventional small-molecule inhibitors that often act by occlusion of the target protein's active site [27]. Studies of PROTACs as a replacement of the UPS in 2001 opened the chemical biology approach to regulate protein function for drug discovery in the current era [4-11]. This concept of drug discovery involves miniature molecules and is the only approach that has the possibility to overcome the widely documented shortcomings of targeted drug therapy [12]. Whereas traditional small molecule inhibitors are prone to off-target effects and drug resistance since they only inhibit target protein activity, PROTACs overcome these problems by degrading target proteins fully. This mechanism increases the selectivity and potency of therapeutic intervention and opens the prospects for using drugs for targets that were previously considered untouchable [13,14]. Because PROTACs are so potent the field of Targeted Protein Degradation (TPD) expanded and went beyond the proteasome [15]. An example is the Lysosome Targeting Chimeras (LYTACs) which translocate the lysosomal degradation pathway to export proteins labeled with a specific organelle targeting signal. Another is the Macroautophagy Degradation Targeting Chimera (MADTAC) platforms the AUTACs and ATTECs that subvert the autophagy pathway. This could help to disrupt organelles and macromolecular complexes with a view of eliminating them [16,17].

In the subsequent decade there were major developments in PROTAC technology based on progress in the chemistry of linkers and in the characterisation of stronger E3 ligase ligands. The field was revolutionized with the advent of small-molecule PROTACs which are more drug-like and have preferable pharmacokinetic characteristics. These progress has boosted the general suitability of PROTACs to numerous target proteins that were once deemed as 'undruggable' [3,4]. Currently, several PROTAC drugs are under clinical trial examination. One of them is ARV-110 [18], that acts on the androgen receptor in the prostate cancer. Another is ARV-471 [19], a selective estrogen receptor antagonist indicated in treating breast carcinoma. Last is FHD-609 in which BRD9 is being targeted in synovial sarcoma [20].

Several PROTACs have thus been designed to target a variety of POIs involved in the development and progression of hematologic malignancies. Some of them are anaplastic lymphoma kinase (ALK) [21], Bcl-xL [22], breakpoint cluster region Abby like (BCR-ABL) [23], brutons tyrosine kinase (BTK) [24], bromodomain containing 4 (BRD4) [25], cyclin dependent kinase-6 (CDK-6) [26], FMS-like tyrosine kinase-3 (FLT Some agents are highly effective agents in eradicating leukemia and cancer cells in test tubes and can be capable of producing the complete remission and excision of tumors in animals [29]. Since their inception, PROTACs have shown the ability to degrade several and diverse target proteins associated with numerous disorders such as cancer, immunological diseases, neurological disorders, cardiovascular diseases and viral infections [30-32]. The degradation of target proteins by PROTACs has been proven to be efficient in sixty cases and two of them are in trial to treat prostate and breast cancer [33,34].

Recent advancements in PROTAC technology have focused on optimizing pharmacokinetics, bioavailability, and delivery mechanisms. The integration of PROTACs with advanced drug delivery platforms, such as nanoparticles (NPs) and lipid-based carriers, has significantly improved their stability and targeted delivery to disease sites has been a significant focus of recent research. These developments enhance the clinical potential of PROTACs and broaden their application across various medical conditions [35]. These efforts aim to improve the pharmacokinetics, bioavailability and targeted delivery of PROTACs. One notable advancement is the use of NPs as delivery vehicles. NPs can protect PROTAC molecules from degradation in the bloodstream, enhance their stability, and facilitate their

targeted delivery to diseased tissues. For instance, lipid-based NPs (LNPs) have been employed to encapsulate PROTACs, thereby improving their solubility and cellular uptake [36].

Furthermore, polymeric NPs have been fabricated so as to address the controlled release of PROTACs for constant therapeutic impact. These NPs can be designed to be sensitive to certain conditions in the tumor microenvironment including pH or enzymatic activity and thus, release the PROTACs in the right location [37]. To this effect, targeted delivery minimizes side effects that arise from the exposure of surrounding healthy cells and tissues as well as improves the effectiveness of the cure. Moreover, with Demdrimers, it has also been possible to fashion more elaborate delivery systems for PROTACs. Oleinik et al. described chemical knockdown method, which they called "ligation to scavenging", as an unconventional method for halting event-based proteolysis. This technique incorporates a ligation to a scavenging system of a tetrazine-functionalized BRD4 PROTAC 79 and PAMAM-G5-TCO to target the epigenetic regulation selectively. Being an efficient intracellular, nonspecific proteasome-targeted macro cationic dendrimer, PAMAM-G5-TCO quickly desorbs free PROTACs by the IEDDA approach. This in turn helps in preventing the degradation of the BRD4 protein and provides control of protein degradation termination. This is a novel chemical strategy that deserves about equal consideration as mechanical disruption methods and offers the possibility of modulating protein disintegration under command [38].

Similarly, the intracellular delivery of PROTACs has been advanced by conjugating them with cell-penetrating peptides (CPPs), thereby extending the capability of PROTACs to intercalate and degrade target proteins in cells [39]. Another interesting advancement is the application of exosomes to deliver PROTACs on target proteins. Exosomes are biomembrane-derived particles that are involved in the exchange of biomolecules across the cell membrane. By using loading PROTACs into exosomes, researchers make use of these vesicles to pierce barriers and deliver therapeutic agents right at the target cell. It may be worth emphasizing that this approach can enhance the therapeutic index of PROTACs, especially for diseases in which targeted delivery to specific cell types is difficult [40].

According to a recent bibliometric analysis retrieved from Pubmed, more than 2,251 papers on PROTAC were published from 1986 to 2024 [41]. Nevertheless, more candidates needed to enroll in clinical trials due to the small number of molecules that showed possible development. When rationally designing new candidates, a clearly defined experimental workflow using default protocols is essential [42]. There are at least 20 PROTACs in the clinical trials by the end of 2022 [43]. The review included clinical trials from 2022 to 2024, as outlined in Table 1.

Table 1. PROTACs in clinical trials.

Clinical trial no.	Sponser	Degrader	Target	Indications	phase	Study Start	Reference
NCT05501769	Arvinas	ARV-471 in combination with Everolimus	ER	Metastatic ER+, HER2- Breast Cancer	I	08.09.2022	[44]
NCT06206837	Pfizer	Vepdegestrant When Given With PF-07220060	ER+/HER2-	Metastatic Breast Cancer	II	19.02.2024	[45]
NCT06125522	Pfizer	Vepdegestrant When Given With Samuraciclib	ER+/HER2	Breast Cancer	II	10.01.2024	[46]
NCT05573555	Pfizer	Vepdegestrant When Given With Ribociclib	ER+/HER2	Breast Cancer	II	01.03.2023	[47]
NCT05548127	Pfizer	ARV-471 When Given With Abemaciclib	ER	Breast Cancer	II	23.02.2023	[48]
NCT05654623	Pfizer	ARV-471 When Given With Fulvestrant	ER+/HER2-	Advanced Breast Cancer	III	03.03.2023	[49]

Data source: https://clinicaltrials.gov [accessed: 06/06/2024]

PROTACs mechanism

The mechanism of action of PROTACs involves the formation of a ternary complex comprising the target protein, the PROTAC molecule, and the E3 ubiquitin ligase. The three steps (Figure 1) in this process are as follows: **Binding**: The targeting ligand of the PROTAC binds to the POI, while the E3 ligase ligand binds to the E3 ubiquitin ligase. **Ternary complex formation**: Upon simultaneous binding, a ternary complex is formed. The efficiency of this complex formation is critical for the subsequent steps. The structure and flexibility of the linker play a crucial role in facilitating this interaction. **Ubiquitination**: The formation of the ternary complex brings the POI in close proximity to the E3 ligase, allowing the transfer of ubiquitin molecules from the E2 ubiquitin-conjugating enzyme to the lysine residues on the POI. This process is mediated by the E3 ligase, which acts as a scaffold for ubiquitin transfer. **Proteasomal degradation**: The polyubiquitinated POI is recognized by the 26S proteasome, a large protease complex responsible for degrading ubiquitinated proteins. The POI is subsequently unfolded and degraded into small peptides, while the PROTAC molecule is released and can engage in additional rounds of degradation [18,50].

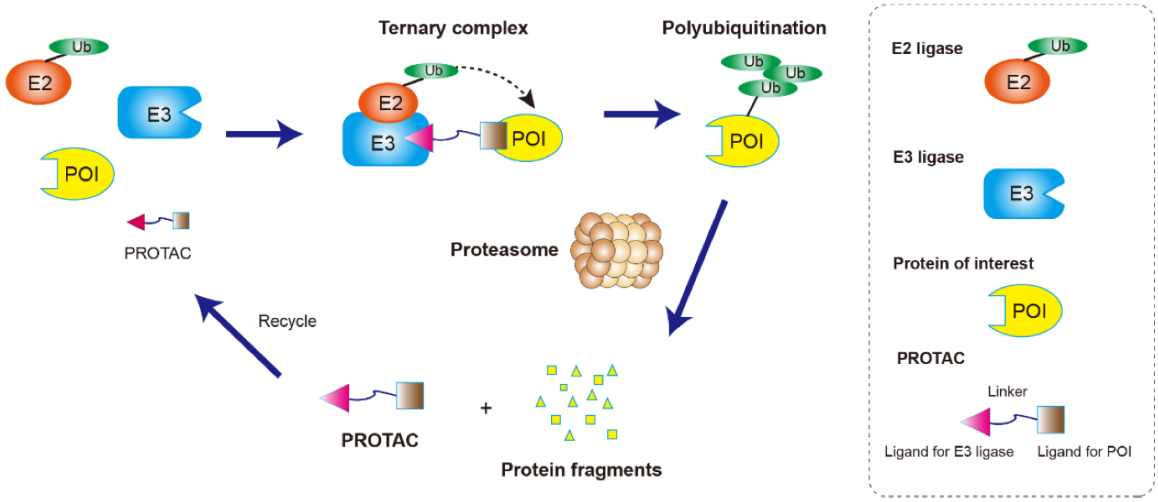

Figure 1. PROTAC mechanism. A PROTAC consists of a ligand that attaches to a protein of interest (POI) and another that connects to the ubiquitin ligase enzyme via a small chemical linker. By forming a POI-PROTAC-E3 ligase ternary complex, the target protein is tagged with ubiquitin groups. The proteasome recognises the polyubiquitination signal and facilitates the protein's breakdown. To facilitate further breakdown cycles, the PROTAC molecule can be recycled. Reprinted under the terms of the Creative Commons Attribution 4.0 International (CC BY) license [1].

The original proposal for PROTACs was made in 2001 by Sakamoto et al. [5]. They developed and synthesized the first bifunctional PROTAC molecule to degrade methionine aminopeptidase 2 (MetAP-2). In its initial form, the PROTAC molecule consisted primarily of peptides. A portion of the PROTAC-1 molecule that directed its attention to MetAP-2 bound to ovalicin, an inhibitor of angiogenesis, and another portion bound to the E3 ubiquitin ligase complex SCF (Skp1-Cullin-Fbox), an IPP derived from the IKB protein that β-TRCP uniquely identifies. Next, it was shown that PROTAC-1 could recruit MetAP-2 and SCFβ-TRCP. Different PROTACs have been created for numerous proteins since the preliminary result, including the BET protein family [5], EGFR [25], and aperiodic cyclin-dependent kinases (CDKs) [51]. The PROTAC approach has also been improved. Many of these proteins are currently being investigated as possible therapeutic targets or linked to cancerous tumors' progression and metastasis. In contrast to other approaches that have been developed to control tumor-related protein expression or function, such as CRISPR, siRNA, small molecule inhibitors, monoclonal antibodies, etc., PROTAC offers numerous benefits, such as a wide range of targets, rapid action, and high specificity in degrading pathogenic variant proteins while leaving normal proteins alone [52,53].

Application of PROTAC

Oncology

As we know, cancer is one of the dangerous diseases which can threat human life. A cancer patient is not restricted to surgical intervention, radiation, or chemotherapy as the only ways to cure. Another essential component of cancer treatment is a targeted treatment and immunotherapy. Such 'un-druggable' sites like KRAS and TP53 for instance, are still unlabeled by effective targeted drugs [54]. The main advantage of PROTAC technology is the shift of focus from "no drug" to "drug. " Affinity to the target protein is critical for conventional targeted drugs. Due to its capability to selectively and weakly interfere with target proteins, the PROTAC degradation agent can address many of the "undruggable" proteomes today, that is, around 80%. Literally it proves to be a lifesaver when conventional targeted therapy is not possible for a particular patient [55]. There are many oncogenic proteins, for example, kinase, transcription factor, and epigenetic enzymes, which play a significant role in carcinogenesis. Several PROs have been demonstrated to be targeted by PROTACs, which destabilise these proteins, hence suppressing cancer cell proliferation and survival. For instance, ARV-110 and ARV- 471 is two PROTACs discovered by Arvinas targeting androgen receptor and estrogen receptor respectively. These receptors are very essential in cases of prostate and breast cancer. These and other PROTACs have been proven to selectively degrade their targets and suppress tumor cells within vitro clinical trials, thus, the innovation has potential to be a new form of targeted cancer therapeutics [56,57].

Leukemia

Several PROTACs have demonstrated high efficacy in leukemic cells by providing a new approach for the degradation of proteins that are associated with the formation of the disease. Leukemia, the cancer of the white blood cells, is generally associated with the mutations that affect intrinsic factors such as the genetic code and protein functions, which cannot be effectively treated with conventional approaches. The problem of synthesising and purifying such proteins can be a concern and therefore PROTAC technology offers a solution that reacts with the cell's own machinery of protein degradation [58].

PROTACs targeting BCR-ABL in chronic myeloid leukemia

Chronic Myeloid Leukemia (CML) is associated with increased activity of BCR-ABL fusion protein that is a consequence of the translocation t(9;22). Conventional therapies for CML, especially TKIs including imatinib, have shown remarkable success for the initial years but increasing resistance resulting from BCR-ABL gene mutations poses a problem. PROTACs targeting BCR-ABL represent a more favorable strategy. For instance, it has been shown that the approach using both BCR-ABL1 kinase inhibition and protein degradation is currently a promising approach in overcoming BCR-ABL1-mediated drug resistance. Targeting BCR-ABL1 for degradation in CML through small molecules is better than targeting it and offers information about CML stem cells [59].

PROTACs in acute myeloid leukemia

Acute Myeloid Leukemia (AML) is another form of leukemia where PROTACs have shown promise. AML is characterized by the rapid proliferation of immature myeloid cells, often driven by mutations in various signaling proteins and transcription factors. Targeting these proteins with PROTACs can induce their degradation and inhibit leukemic cell growth. One study described an oral activity BRD9 PROTAC C6 by recruiting the highly efficient E3 ligase. C6 exhibited superior oral activity, with a Cmax value of 3436.95 ng/mL. These findings demonstrated that C6, as a novel BRD9 PROTAC with remarkable pharmacodynamic and pharmacokinetic properties, had the potential to be developed as a promising therapeutic agent for AML treatment [60]. An internal tandem duplication (ITD) in the juxta-membrane domain is a common mutation in FLT-3, a therapeutic target in acute myeloid leukaemia (AML) [61,62]. One study found that a chemical that triggers the degradation of the FLT-3 ITD mutant at low nanomolar doses was produced by converting the FLT-3 inhibitor quizartinib into a PROTAC. Compared to the warhead alone, the PROTAC can limit cell growth with greater potency and fewer off-target kinases inhibited. The increased level of apoptosis induction suggests that the FLT-3 ITD protein

has nonkinase roles. This explains why the PROTAC's kinase inhibitory action is slightly reduced while the antiproliferative activity remains unchanged. The PROTAC can also induce the degradation of FLT-3 ITD in living organisms. Based on these findings, FLT-3 ITD degradation could be a promising therapeutic target [27,63].

Neurodegenerative diseases

There are currently no effective therapies or drugs for several refractory diseases, including neurodegenerative ones such as Alzheimer's, Parkinson's, progressive supranuclear palsy, and frontotemporal dementia. Conventional medications have failed miserably at targeting a large number of neurodegenerative disease-causing proteins. None of the compounds that reduced the rate of amyloid-b (Ab) accumulation have been authorized for usage in clinical trials, and numerous therapeutic trials for Alzheimer's disease have been unsuccessful. Novel developments in PROTACs provide an alternative perspective on this problem [64,65]. So far, PROTACs have effectively targeted a wide variety of proteins associated with neurodegenerative diseases, such as a-Synuclein [66], mHTT [67], GSK-3 [68], LRRK2 [69], Tau [70], TRKA [64], and TRKC [71].

PROTACs in Alzheimer's disease

Alzheimer's disease is a type of dementia disorder in which people experience progressive cognitive decline due to deleterious deposition of the amyloid beta (Aβ) and neurofibrillary tangles (tau protein) in the brain. PROTACs have been revealed as an effective therapeutic strategy for selectively and ubiquitinate these pathological proteins [72]. Targeting Tau and Amyloid Precursor Protein (APP) are considered to be of paramount importance in the development of AD. Tau protein leads to the formation of neurofibrillary tangles in AD which is toxic to the neurons. Antibodies against IST tau would decrease ist levels and prevent tau aggregation and formation of neurofibrillary tangles, which presumably should attenuate disease progression. In one study, Authors used PROTAC to selectively degrade tau in neuronal cells with reduced tau aggregation and toxicity. In this case, the selective degradation of tau has the potential to alleviate tau-mediated neurodegeneration [73]. Specific for the elimination of intracellular tau proteins, Chu et al. synthesized a small peptide, called TH006, which is a PROTAC that enhanced poly-ubiquitination of its target. TH006 also had effects on tau decrease in primary neuron cells and a mouse model of AD, and the inhibition of cytotoxicity induced by Aβ12 [74]. Wang et al.'s similar work contributes to this conclusion. Another compound was synthesized and it was confirmed to enhance the tau degradation via UPS and lessen the cognitive impairments in Alzheimer-like models [70], all of which indicating that protein degradation is advantageous in order to combat aggregation problems [75].

PROTACs in Parkinson's disease

Parkinson's disease (PD) is an ongoing neurodegenerative and movement ailment that has been established by the degeneration of the dopaminergic neurons that are found in the substantia nigra coupled with the α-synuclein proteins. In PD, PROTACs are employed to degrade α-synuclein directly, bringing down the toxic levels of the protein [76-79]. One of the protein aggregates evident in PD is alpha synuclein which is known to be damaging and fatal to neurons. Recently, Sun et al. established a PROTAC for degradation of α-synuclein in dopaminergic neurons and alleviation of their neurotoxicity. This work also showed that via the generation of PROTACs, α-synuclein aggregation as well as ist-associated neurotoxicity can potentially be managed [31]. LRRK2 gene polymorphism is perhaps one of the most popular and best-studied genetic factors contributing to PD. Here, we shown that LRRK2 can be targeted for degradation using a well-studied technique called proteolysis-targeting chimeras (PROTACs) which provides a new therapeutic strategy. These mutations are identified mostly in LRRK2 gene but most especially the G2019S mutation which increases the kinase activity of the protein and is toxic to neurons hence causing Parkinson's disease. Small molecule chimeric inhibitors of LRRK2 have been explored and while they have their limitations such as partial inhibition and off target toxicity. PROTACs on the other hand, which use the cell's ubiquitin–proteasome system to target their protein and degrade it selectively, provides an answer to these limitations. In the recent past, much

research effort has been directed towards designing and fine tuning PROTACs for the selective degradation of LRRK2. Liu et al. described the research done in identifying LRRK2 proteolysis targeting chimeras (PROTACs) to devise degrader XL01126. Altogether, these experiments demonstrate that XL01126 can be considered as an appropriate degrader probe for dissecting the non-catalytical and scaffold functions of LRRK2 in vitro and in vivo [80].

PROTACs bioavailability: Challenges and strategies

It is the molecular structure that distinguishes PROTACs from traditional chemo-drugs as far as their physicochemical properties are concerned. This is because, although PROTACs display a lot of promise in the treatment of many diseases, the bioavailability is a major barrier. The issue of providing sufficient bioavailability to drugs derived from these types of compounds is difficult because of their size, multifunctionality and other chemical properties [81-84].

Factors affecting PROTAC bioavailability

Molecular size and complexity

PROTACs are bispecific molecules consisting of two functional ligands linked by an intervening chain. This structure often leads to high molecular weights and molecular mass which in a way negatively affects their absorption and distribution. In general, PROTACs have MWs between 800 to 1,200 Da, which are larger than traditional small molecule drugs. This size can sometimes hinder their entrance into the cellular matrices via passive diffusion [85]. Moreover, several polar groups in PROTACs could enhance the hydrophilicity, which subsequently results in a decrease in membrane permeability [13].

Solubility

The appreciable solubility of PROTACs in biological fluids makes the absorption and bioavailability functions a key factor here. PROTACs are also insoluble in aqueous media and therefore poorly dissolve in gastrointestinal tract and can be absorbed [86-88]. Solubility of PROTACs may be further improved by complicated formulation strategies, that is utilizing solubilizers or employing proteolysis-targeting chimeras with the help of nanocarrier systems [89]. Most PROTACs face issues related to intracellular delivery and having a low bioavailability. Many approaches have been attempted to boost their performance by prognostically modifying the scaffold of PROTACs to remove their relatively poor properties mainly due to the chemical characteristics of PROTACs. Klein et al., further explaining the method of construction of the PROTACs, believed that one way of lowering the amount of intramolecular hydrogen bond donors and improving the cell permeability of the PROTACs might be done by the use of ester groups to replace the amide groups in between the linkers and the warheads [90]. Another correlation that was discovered regards the increase of cell permeability and the decrease of molecular polarity where large linkers with lipophilic side chains or the substitution of more polar E3 ligase recruits might be beneficial [91,92]. However, it is important to remember that as TPSA is directly related to water solubility raised cell permeability by reduced molecular polarity can affect the water solubility of PROTACs and their pharmacokinetic response. This has called for more research in the development of theories in this area.

Stability

The stability of PROTACs present in any of the body fluids also determines the problem of bioavailability. As a result of this, PROTACs may be chemically unstable and could be degraded in the stomach before getting absorbed [15]. In the same regard, enzymatic Degradation is an issue because PROTACs are vulnerable to being broken down by proteolytic enzymes hence limiting the quantity of the active drug in the systemic circulation [93].

Strategies to improve PROTAC bioavailability

Nantechnology-based delivery systems

NPs represent a promising approach to improving the solubility and, therefore, the efficacy of PROTAC compounds. The encapsulation of PROTACs in NPs may enhance solubility, stability, and bioavailability besides shielding the complex from degradation and elimination within the cells. These nanoparticle based delivery systems are capable of releasing the PROTACs in a controlled and more informed manner to influence the positive change in the pharmacokinetic characteristics of the PROTACs. Some of the delivery strategies include antibodies, small-molecular targeting agents, organic NPs, liposomes, polymeric NPs, inorganic NPs, and lipid NPs of PROTACs, as illustrated in Figure 2 [84].

NPs and PROTAC molecules are at the crossroads between nanobiotechnology and targeted protein degradation for therapeutic uses. However, issues such as the poor solubility and cell permeability characterise traditional PROTACs as well as off target effects. To overcome these limitations, Wang and colleagues have also turned to the functionalization of PROTACs with nanotechnology leading to nano-PROTACs. Some of these nanostructured PROTACs can improve issues concerning the delivery, stability, as well as the selectivity of PROTACs. However, the nano-PROTACs can be partly modified to enhance solubility and bioavailability to enable enhanced targeting of disease-protein. Thus, employing NPs as carriers helps to obtain targeted delivery of PROTACs into the body without causing multiple side effects and enhance the overall efficiency of the treatment [94]. These developments point to the possibilities of using nanoparticle based delivery systems in extending the potential of PROTAC bioavailability and its actual application in therapies. The application of NPs in the formulation of drugs has been well illustrated in the improvement of solubility and pharmacokinetics. Compared to drug molecules, NPs are relatively bigger in size, thus once the drugs are loaded into NPs the solubility of the drugs is solely determined by that of the NPs [97-99]. Dendritic targeted passive targeting, also known as enhanced permeability and retention (EPR), occurs in the case of NP within certain size ranges that are preferably taken up in tumor tissues because it lacks functional lymphatics and encompasses abnormally permeable vasculature [100–102].

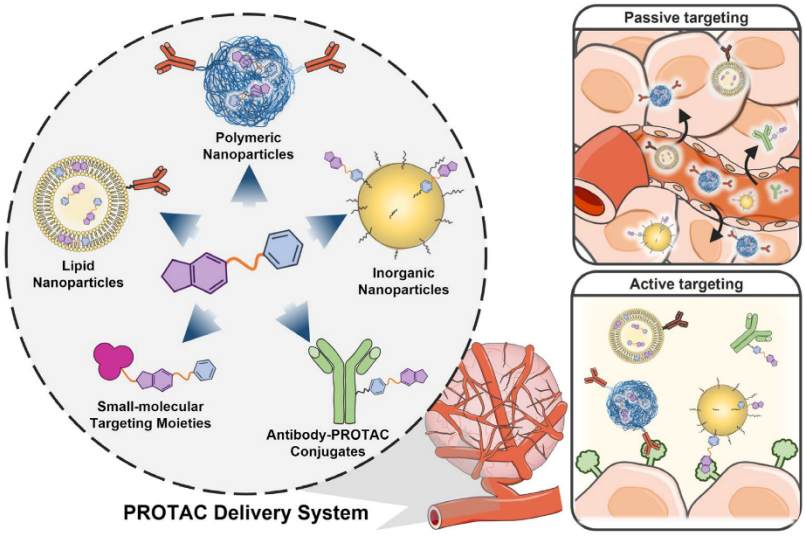

Figure 2. Carriers for PROTAC delivery. Through passive and active targeting approaches, they control the pharmacokinetic features of PROTACs while simultaneously raising their accumulation levels in tumours. Reprinted under the terms of the Creative Commons Attribution 4.0 International (CC BY) license [84].

The pharmaceutical NPs are involved in delivery of drugs as are other NPs in the pharmaceutical field. There are inorganic and organic forms of NPs; these are further sub-divided into lipid and polymeric forms. From among those, polymeric NPs can boast of being one of the most standard and efficient systems for the delivery of drugs. In these respects of, these NPs have the ability of presenting a broad spectrum of pharmacological/physicochemical properties through adjustment on monomers

and polymeric chains [103]. Among the polymeric based drug delivery systems, which has been approved by the FDA, the most common is, block copolymer of poly (ethylene glycol) (PEG) and poly (D, L-lactide-glycolide) (PLGA) [65]. The research work done by Sarawat et al. has, therefore, resulted in a significant success in the treatment of pancreatic cancer. They developed an inert delivery approach for lipophilic ARV-825, a PROTAC degrades bromodomain 4 (BRD4) that is nestled within PEG-PLGA NPs for parenteral administration. The outcome was dramatic and showed the usefulness of the ARV-Nanoparticle on pancreatic cancer cells. It provoked cytocidal effect, cell death, and an anti-clonogenic activity. The pro-apoptotic protein cleaved caspase-3 was also induced while the anti-apoptotic protein Bcl-2 was significantly reduced together with BRD4 and c-Myc. Probably, the most important proof of its efficiency was the decrease of cell viability of 3D pancreatic cancer tumor spheroids after treatment with ARV-NP. It is opening the way to a new approach to pancreatic cancer treatment that holds much promise [104].

Gao et al. engineered a polymeric PROTAC (POLY-PROTAC) nano platform for tumor-targeted degradation of the bromodomain and extra terminal (BET) protein BRD4. First, they synthesized four von Hipel-Lindau (VHL)-based small molecular PROTACs. Then, they designed a series of reduction-activatable POLY-PROTACs and self-assembled them into micellar NPs for systemic PROTAC delivery (Figure 3a). Then, a dibenzocyclooctyne (DBCO)-loaded pre-targeted NP was engineered to enhance intratumoral accumulation and retention of azide-modified POLY-PROTAC NPs via in situ click reaction. Upon internalization into the tumor cells, the POLY-PROTAC NPs release the PROTAC payload via glutathione (GSH)-mediated reduction of the disulfide bond (Figure 3b). Outcomes demonstrated that the POLY-PROTAC NPs synergistically induce apoptosis of tumor cells when combined with photodynamic therapy (PDT) in a mouse model of MDA-MB-231 breast cancer (Figure 3c). This study might provide a generalizable nano platform for tumor-specific PROTAC delivery and potentiated cancer therapy [105].

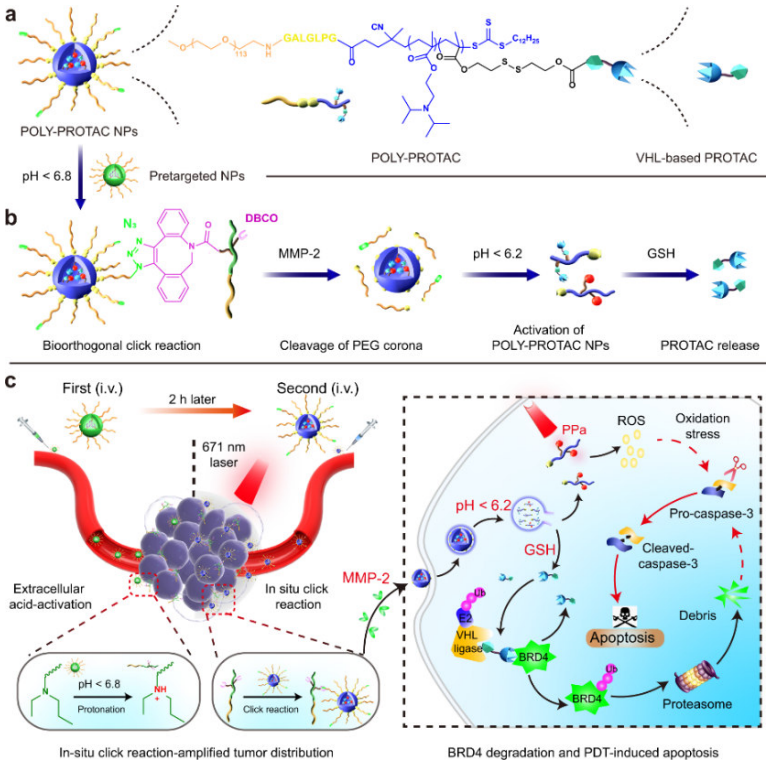

Figure 3. Schematic illustration of the 9iorthogonal POLY-PROTAC NPs for tumour-specific protein degradation and precise cancer therapy. [a Cartoon illustration of the azide-functionalized biorthogonal POLY-PROTAC NPs. POLY-PROTAC was engineered by integrating an MMP-2-liable PEG chain, an acid-activatable DPA moiety and a reduction-sensitive disulfide spacer. B Schematic illustration of the extracellular acidity-triggered click reaction between POLY-PROTAC and DBCO-loaded pretargeted NPs and sequential activation of POLY-PROTAC in response to the extracellular enzyme and intracellular acidic/reductive microenvironment. C In situ click reaction-promoted protein degradation and combinatorial cancer therapy with POLY-PROTAC NPs. The POLY-PROTAC NPs showed tumour-specific accumulation and retention via a biorthogonal click reaction with the pretargeted NPs and cleavage of the PEG corona in the tumour mass. The POLY-PROTAC NPs were then internalised into the tumour cells for BRD4 degradation and combination therapy with PDT]. Reprinted under the terms of the Creative Commons Attribution 4.0 International (CC BY) license [105].

In another study, Xu et al. created an RCNprotac, a nanomicelle that responds to X-ray radiation to combat cancer. A 141.80 ± 5.66 nm nano micelle was self-assembled after a previously reported small molecule PROTAC (MZ1) was covalently coupled to hydrophilic PEG via a carbon chain that contained diselenide bonds. Due to its improved permeability and retention effect, RCNprotac—which at first showed no bioactivity in circulation because the hydroxyl group on the E3 ubiquitin ligand component was occupied-could accumulate at the tumor site. By breaking the radiation-sensitive diselenide

linkages, X-ray radiation released MZ1 for the targeted degradation of the tumor BRD4 protein. A decrease in BRD4 protein level occurred, making the tumour more radiosensitive. Synergistic increases in anticancer effects were demonstrated in vitro and in vivo by RCNprotac. Finally, this X-ray-responsive PROTAC nano micelle may give an innovative approach to X-ray-activated spatiotemporally controlled protein degradation and BRD4 proteolysis improved tumour radiosensitivity, which anticipates the eventual success of cancer research [106].

The primary finding on the cancer-fighting potential of the HSP90 degrader BP3, which was synthesized using the PROTACs principle, was conducted by Jiang et al. [107]. Unfortunately, its insolubility in water and high molecular weight limited its practical use. This research aimed to enhance these features of HSP90-PROTAC BP3 by incorporating it into NPs made of human serum albumin (BP3@HSA NPs). The findings revealed that BP3@HSA NPs had a consistent spherical form, a size of 141.01 ± 1.07 nm, and a polydispersity index of less than 0.2. Moreover, compared to free BP3, BP3@HSA NPs were more or less taken by breast cancer cells and exhibited a stronger inhibitory impact in laboratory tests. It was also shown that BP3@HSA NPs may break down HSP90. From a mechanistic standpoint, BP3@HSA NPs' enhanced capacity to induce cell cycle arrest and apoptosis was associated with their enhanced inhibitory effect on breast cancer cells. Additionally, BP3@HSA NPs demonstrated enhanced pharmacokinetic characteristics and demonstrated more robust tumor reduction in mice. These results highlight the potential of encapsulating hydrophobic HSP90-PROTAC BP3 NPs in human serum albumin to enhance BP3's antitumor activity and safety significantly.

Amphiphilic lipids are the building blocks of liposomes and lipid NPs, which form nanostructures through self-assembly in water. Their increased biocompatibility and potential for mass manufacture make them an appealing alternative to drug carriers based on polymeric or inorganic NPs [108]. The loading process for lipid NPs is straightforward, and they can accommodate substantial amounts of various medicinal chemical compounds. The size and shape of lipid NPs are influenced by several variables, including lipid chemical content and structure and development techniques [109]. However, the morphological parameters of lipid NPs are not the only aspect of interest. They are meticulously controlled by the properties of the payloads [110-113], a factor that underscores the significance of the work in the pharmaceutical and medical fields. These properties profoundly impact the drug loading/releasing patterns, physiological stability, and pharmacokinetics.

Research led by Rathod's team has strongly emphasized delivering lipid-based PROTACs [87]. After studying the anticancer activity of a protein degrader known as the Bromodomain and Extra-Terminal motif (BET) in both sensitive and vemurafenib-resistant melanoma, they conducted pre-formulation studies and formulation development. Rather than inhibiting the BRD4 protein, the ARV-825 (ARV) molecule, developed using PROTAC technology, degrades it. The development of ARV-SNEP, or ARV-loaded self-nano emulsifying preconcentrate, was contingent upon thorough pre-formulation experiments. ARV exhibited hydrolytic breakdown dependent on pH and extremely low water solubility (<7 μg/ml). ARV is a substrate of CYP3A4 but not of the P-gp efflux pump, according to investigations using human liver microsomes and a CaCO-2 cell uptake assay. Enhanced ARV solubility in a range of water- and bio-relevant environments was accompanied by the formation of 45.02 nm nano globules by optimized ARV-SNEP, which exhibited a zeta potential of -3.78 mV. Crucially, when tested on vemurafenib-resistant melanoma cells, ARV demonstrated promising cytotoxicity, anti-migration, and apoptotic effects. The development of ARV-SNEP, with its unique properties and demonstrated efficacy, may herald a new therapeutic paradigm for drug-resistant melanoma.

An emerging class of anticancer compounds, PROTACs, has substantial solubility problems. Much research has been conducted into colloidal systems based on lipids, such as nanostructured lipid carriers, for these highly lipophilic compounds. Scientists used the melt emulsification method to create an ARV-825 loaded PEGylated nanostructured lipid carrier (AP-NLC) of BRD4 protein degrading PROTAC that targets non-small cell lung carcinoma. The ARV-825 was stabilized with the use of Precirol® ATO5 as the solid lipid and Captex® 300 EP/NF as the liquid lipid. The results showed a hydrodynamic diameter of 56.33 ± 0.42 nm, a polydispersity index of 0.16, and a zeta potential of -21 ± 1.24 mV. ARV-825 and AP-NLC demonstrated antitumor efficacy in cultured cell migration and colony

formation assays. Researchers observed an almost 38% and 50% apoptotic cell population following ARV-825 and AP-NLC therapy, respectively. An immunoblotting experiment showed evidence of complete inhibition of BRD4 and c-Myc protein expression for AP-NLC. To conclude it all, BRD4 PROTAC and its lipid nanoparticle were proven efficient against non-small cell lung cancer (NSCLC) when they significantly reduced the growth of multicellular 3D spheroid of A549 cells. The increased red fluorescence observed across the spheroid surface provides more evidence of AP-NLC's enhanced penetrating and cell-killing capabilities, highlighting the promising potential of this new delivery system in clinical applications [114].

In order to combine a synergistic cytotoxic ratio, a chimaera targeting BRD4 proteolysis (ARV-825) and nintedanib co-loaded PEGylated nanoliposomes (ARNIPL) were produced. Neither of the molecules is soluble in water at all. A modified hydration technique containing citric acid was employed to enhance the loading of both compounds into liposomes. At 4 °C, ARNIPL showed physical stability for one month and demonstrated an encapsulation efficiency of over 90% for both medicines, with an average particle size of 111.1 ± 6.55 nm. A375R, a vemurafenib-resistant cell line, demonstrated increased cytotoxicity, apoptosis, and down-regulation of target proteins BRD4 and c-Myc in response to both drugs and ARNIPL. ARNIPL considerably reduced the clonogenic potential and vasculogenic mimicry of A375R. With ARNIPL, tumor growth suppression and a significant decrease in TGF-β1, a key regulator of immune response and tumor progression, were observed in 3D spheroids. The results showed that ARNIPL has the potential to be a successful treatment for vemurafenib-resistant melanoma, with the added benefit of modulating the immune response [115].

Inorganic NPs, such as silica, gold, iron oxide, and quantum dots, are potential candidates for PROTAC delivery, offering unique properties distinct from organic NPs. The meticulous control over their morphology and size distribution, coupled with their rigid structure that minimizes the risk of drug leakage at off-target sites [116], presents a promising avenue for drug delivery. Gold NPs (GNP), in particular, hold great promise as drug carriers thanks to their bio inertness, well-established surface modification method, and versatility in providing additional functionalities, instilling optimism in their potential. A drug delivery system based on gold nanoparticles (GNPs) was developed in the study by Y. Wang et al. to deliver PROTACs to target Anaplastic lymphoma kinase (ALK). The Cer/Pom-PEG@GNPs, which are pegylated GNPs loaded with ceritinib and pomalidomide molecules, demonstrated excellent stability in various mediums as tested. There was a dose- and time-dependent reduction in ALK fusion protein levels and particular inhibition of NCI-H2228 proliferation caused by the GNP conjugates. Researchers found that Cer/Pom-PEG@GNPs could break down intracellular ALK fusion proteins with only a few side effects and could be used to treat patients not responding to ALK inhibitors. The Cer/Pom-PEG@GNPs nano-drug carrier can deliver medications to tumor areas in vivo with high precision, allowing for sustained circulation [117]. According to another study, a supramolecular gold(I)-thiol-peptide complex (Nano-MP) was designed to incorporate proteolysis recalcitrance, cellular internalization, and glutathione-triggered release. The complex was nanoengineered to target a tumor-driving protein, MDMX, for degradation. To enhance tumor targeting, nano-MP was coated with a pH-responsive macromolecule called polyacryl sulfydryl imidazole (PSI). Results demonstrated that Nano-MP@PSI induced the MDMX degradation by ubiquitination and subsequently restored the anti-cancer function of p53 and p73 [118]. Further investigation is essential to evaluate the effectiveness of different inorganic NPs for PROTAC delivery beyond gold, which has not been explored previously.

Targeting moiety-functionalized NPs

Thus, targeting moiety-functionalized NPs is a current state of the art in the targeted drug delivery, proving to be especially valuable in the case of PROTACs. These NPs are designed to be functionalized with specific ligands or antibodies at the surface, which can specifically react with receptors that are overexpressed on the surface of target cells, including tumour cells. This functionalization improves the targeting ability and the specificity in which drug loaded-NPs lodge in the required site, reducing the unwanted interaction with other tissues, and subsequently improves on the effectiveness of the

administered drug [95,96]. The exposure of PROTACs to tumor sites and the proper management of their undesired pharmacokinetic profile have been enhanced by linking PROTAC molecules to antibodies or aptamers. However, the loading capacity of mAb-PROTAC is restricted. Due to the tendency of antibodies to cluster or to be rapidly cleared in physiological circumstances if there are a large number of drug molecules conjugated to the antibodies, traditional ADCs allow no more than four drug/antibody ratios to ensure the optimal potency of the conjugate [101,119,120]. Since PROTACs, generally possess a higher molecular weight and are less soluble in water compared to chemo-drugs, the proportion of PROTACs to antibodies should be kept to not more than three. In turn, owing to their chemical structure, NPs can incorporate a rather large number of PROTACs in their structure. This is because they are comparatively less sensitive to the loading amounts than the antibodies and, therefore, make suitable PROTAC carriers.

Moreover, the incorporation of active targeting moieties on the nanoparticle surface can enhance tumor accumulation of PROTACs, suggesting a potential strategy for improving the efficacy of cancer treatment [84]. In one study, The nanoprecipitation approach was used to load the BRD4-degrading PROTAC (MZ1), which consists of a BRD4-binding ligand and a VHL E3 ligase recruiter, into poly(D, L-lactide) (PLA) NPs. The polyethyleneimine (PEI) layer was conjugated with trastuzumab, and the particles were developed with a size of around 114 nm. The nanoparticle was found to have a loading amount of 0.5% MZ1, and the loaded MZ1 was released gradually in solution. Endocytosed MZ1 induced death in HER2-expressing cancer cells linked with BRD4 deficiency, and trastuzumab on the particle surface greatly improved nanoparticle internalization [121].

Subsequently, He et al. suggested a therapy strategy that combines doxorubicin (DOX) with the BRD4 PROTAC degrader ARV-825 (ARV) using a self-assembly process and a nanoparticle modified with Cyclo (Arg-Gly-Asp-d-Phe-Lys) (cRGDfk) peptides, cRGD-P. Conformational changes made possible by molecular dynamics simulations allowed cRGD-P to offer interaction sites for optimal co-loading of DOX and ARV. On average, the cRGD-P/ARV-DOX was 39.95 nm in size and had a zeta potential of -0.25 mV. After being stimulated with cRGD-P/DOX, glioma cells showed increased BRD4 expression, which supports one of the potential mechanisms of DOX resistance and the synergistic tumor inhibitory impact of BRD4 degrading ARV coupled with DOX. Through glioma cell cycle arrest in the G2/M phase and activation of tumor cell apoptosis-related pathways, such as triggering a cascade of caspases, downregulating Bcl-2, and upregulating Bax, the study found that DOX and ARV combined in the cRGD-P nanoparticle system synergistically suppressed tumor growth. By enhancing tumor apoptosis, reducing tumor proliferation, and decreasing tumor angiogenesis in vivo, the cRGD-P/ARV-DOX system successfully limited gliomas' heterotopic and orthotopic growth. More effective and safer combination therapy for glioma may be possible with the cRGD-modified nanoparticle that co-delivers DOX and ARV [122].

Another type of PROTAC carrier that can be used for active cancer targeting is lipid NPs. In an effort to combat hepatocellular cancer, A. Saraswat et al. investigated the use of galactose-decorated liposomes to transport PROTAC [123]. In order to study the anticancer effectiveness of GALARV for targeted delivery in hepatocellular carcinoma, they created asialoglycoprotein receptors (ASGPR) directed nanoliposomes that included a new BRD4 protein-targeted PROTAC, ARV-825 (ARV) (GALARV). In vitro studies on hepatocellular carcinoma cells demonstrated that ARV and GALARV (with a size of 93.83 ± 10.05 nm) caused cytotoxicity and apoptosis. Compared to non-targeted nanoliposomes (~3 fold) and ARV alone (~4.5 fold), GALARV had a significantly greater intracellular concentration of ARV, exhibited good physical stability, and exhibited almost no hemolysis. There was a significant decrease in the levels of target BRD4, oncogenic c-Myc, apoptotic Bcl-2, and survivin proteins, as shown by immunoblotting. Notably, 3D hepatocellular carcinoma tumour spheroids treated with GALARV showed markedly reduced cell viability and death. Based on these findings, GALARV appears to be an innovative nanotherapeutic strategy for hepatocellular carcinoma that actively targets PROTACs.

PROTACs in RNA viral infections

Viruses are minute, non-cellular microorganisms that severely threaten human health and the global economy [124]. Viruses can have a whole genome and a variety of proteins, or they can have infectious RNA (viroid and virusoid) [125]. Several viruses have recently produced major epidemics, accounting for more than 70% of infectious disease cases. The current standard of care for preventing and treating human viral infections combines antiviral drugs and vaccines [126,127]. Unfortunately, existing antiviral therapy techniques face increasing resistance from newly emerged viruses, and vaccines are not always effective against new or modified viruses [128]. This highlights the urgent need for innovation in targeting or vaccination techniques and the identification of new pharmacological targets to develop effective antiviral therapeutic approaches. Above, we covered how PROTAC technology has been studied extensively for cancer-related targeted protein degradation of POI. Recently, however, much more information has been available about the function of PROTACs as antiviral medicines. Antiviral treatment techniques based on PROTAC have lately been investigated in various ways to improve resistance profiles. A potent antiviral medication that can withstand the present and future threats of new and re-emerging viral pandemics may be conceivable with the help of these innovative methods [129]. These advancements in antiviral therapy hold great promise but also come with challenges, such as safety and efficacy. Researchers and healthcare professionals must stay updated on these developments and contribute to the ongoing discussions and research in this field.

PROTAC: novel vaccine strategy

Developing effective antiviral vaccines is not without its challenges. Preventing the transmission and infection of viral infections by triggering the host antiviral innate immune response is a vital function of these vaccines, which are expected to work similarly to antiviral drugs [130,131]. Various strategies can be used to generate live-attenuated virus vaccines; these include cold-adapted live-attenuated influenza vaccines, codon-deoptimized viruses, premature termination codon-harboring viruses, hyper-interferon-sensitive viruses, and viral-protein-altered viruses. Live-attenuated virus vaccines are among the most effective and proven preventative measures. However, most of these existing methods result in live-attenuated vaccines, which are linked to a considerable or even entire loss in safety and effectiveness [132]. Vaccines are developed by using microorganisms' toxins or surface receptors, which mimic their identity within the host body. These vaccines, when administered, stimulate an immune response upon the detection of a new foreign item in the body, thereby assisting in the development of humoral immunity. The classification of vaccines includes attenuated, inactivated, toxoid, subunit, conjugate, heterotypic, and genetic types [132,133].

Researchers have demonstrated the effectiveness of a novel PROTAC-based approach in degrading viral proteins. This approach involves fusing a removable proteasome-targeting domain (PTD) to eight viral proteins (M1, PB2, PB1, PA, NP, M2, NEP, and NS1) to create PROTAC viruses. The PTD, containing a proteasome-targeting peptide, ALAPYIP, and a tobacco etch virus cleavage site (TEVcs) linker, ENLYFQG, were individually used to generate PROTAC viruses in co-cultured HEK293T-TEVp/MDCK-TEVp cells. The putative PROTAC viruses were then amplified in MDCK-TEVp cells, and their production and infectivity were verified by the cytopathic effects (CPEs) caused by viral infection. M1-PTD caused evident CPE in MDCK-TEVp cells, whereas no CPE was detected for PB2-PTD, PB1-PTD, PA-PTD, NP-PTD, M2-PTD, NEP-PTD or NS1-PTD. Notably, the CPE caused by M1-PTD was observed only in MDCK-TEVp cells and not in the conventional MDCK.2 cells, providing strong evidence for the effectiveness of TEVp-dependent M1-PTD. Additionally, the group examined the ability of M1-PTD to elicit an immune response in ferrets and mice. They found that the titers for HI (Hemagglutinin Inhibition), NT (Neutralisation), HA (Hemagglutinin), and internally conserved nucleoprotein antibodies were significantly higher than those of individuals who had been immunized with inactivated influenza vaccine (IIV) or cold-adapted influenza vaccine (CAIV). As a result of the improved presentation of degraded viral peptide antigens by MHC molecules, it was additionally observed that the T-cell immune response was robust and competent [134].

PROTAC viruses could be an excellent candidate for a vaccination. An ideal vaccination can achieve a level of attenuation in the host that is suitable for safety while at the same time maintaining a robust immunogenicity in cell lines [135,136]. By utilizing the degraded viral peptides produced by the proteasomal degradation pathway, PROTAC can stimulate an effective immune response, which is in contrast to conventional methods of vaccine manufacture. For this reason, PROTAC technology has emerged as a significant choice for producing safer and more effective vaccinations.

Prodrug approaches

Prodrugs are bioreversible, inactive drug substances that become active and can be metabolized to functional parent drugs in the human body [137]. Thus to optimize the oral bioavailability and therapeutic effect lipophilic prodrugs are used to overcome biopharmaceutical, pharmacokinetic and pharmacodynamics problems like chemical stability, poor solubility, non-site specificity, extensive metabolism, crossing biological barriers, utilizing enzymes of the body, toxicity, and compliance problems due to un-acceptable taste or odour [154]. The prodrug technique can be employed to optimize new chemical entities, and also to improve the qualities of already marketed drugs. Though the prodrug approach was a late stage optimization strategy, it has been tried right from the onset of research and development process from Discovery stage. There is the use of a new chemical entity when producing a prodrug, but it is cheaper than developing a new drug molecule from scratch. The higher efficiency (as compared to the parent medicine) tends to reduce the time taken for drug development, which may lead to saving costs, time and effort [139-141].

There are significant concerns over the possible toxicity of the method due to the uncontrolled degradation of proteins and undesired ligase-mediated off-target effects, even though PROTACs have become viable therapeutic approaches. The potential for toxicity and adverse effects could be reduced through the precise manipulation of the degrading activity of PROTACs. As a consequence of this, a significant amount of work has been put into the creation of cancer biomarker-activating prodrugs of PROTACs [142]. Using a bioorthogonal prodrug technique (called click-release "crPROTACs"), Chang et al. could activate PROTAC prodrugs on-target and selectively release them into cancer cells. Two inactive PROTAC prodrugs, TCO-ARV-771 and TCO-DT2216, were rationally developed by attaching a bioorthogonal trans-cyclooctenes (TCO) group to the ligand of the VHL E3 ubiquitin ligase. The tetrazine-modified RGD peptide, c(RGDyK)-Tz, activates the PROTAC prodrugs for click-release. This enables the targeted degradation of proteins of interest (POIs) in cancer cells, as opposed to noncancerous normal cells, by targeting the integrin $\alpha v\beta 3$ biomarker in cancer cells. The results demonstrated that this technique proves to be viable by selectively activating PROTAC prodrugs in an integrin $\alpha v\beta 3$-dependent way. This activation process then generates PROTACs, which break down POIs in cancer cells. An all-encompassing, abiotic method of targeting cancer cells via the ubiquitin-proteasome pathway could be the crPROTAC strategy [142].

The polymer-conjugated PROTAC prodrug platform, as suggested by Zou et al., offers a safe and effective approach for tumor-targeted administration of the most prevalent von Hippel-Lindau (VHL)- and cereblon (CRBN)-based PROTACs, specific codelivery of a degrader, and conventional small-molecule drugs. The activated, self-assembling PROTAC prodrug NPs demonstrate their ability to target tumor cells and release free PROTAC for targeted protein degradation. In a mouse model, the PROTAC prodrug NPs effectively degraded bromodomain-containing protein 4 (BRD4) or cyclin-dependent kinase 9 (CDK9), resulting in a more efficient regression of MDA-MB-231 breast tumors with lower systemic toxicity. These findings underscore the versatility of PROTAC prodrug NPs as a promising approach for the codelivery of chemotherapeutics and PROTACs, thereby enhancing anticancer efficacy and combination advantages [143].

In order to make HNSCC tumors more sensitive to radiation therapy (RT), Zhang et al. have created a nanosensitizer (RPB7H) by combining NPs of hafnium dioxide (HfO2) with a PROTAC prodrug (BPA771). When administered intravenously, RPB7H NPs bind to neuropilin-1, which is overexpressed in tumors, and then they aggregate in tumor tissue, where they internalize into tumor cells. By increasing oxidative stress, DNA damage, and X-ray deposition, HfO2 NPs make RT more effective. At

the same time, RT-induced H2O2 secretion can activate BPA771, which degrades bromodomain-containing protein 4 (BRD4) and inactivates RAD51-associated protein 1 (RAD51AP1), stopping RT-induced DNA damage repair. The results showed that in a mouse model of head and neck squamous cell carcinoma (HNSCC), the tumor growth might be efficiently regressed by combining these nanosensitizers with X-ray irradiation. The results suggest that targeting the BRD4-RAD51AP1 axis with a PROTAC prodrug-based radiosensitization strategy could be an excellent way to improve the efficacy of radiation therapy (RT) and other treatments for head and neck squamous cell carcinomas [144].

There are some potential advantages when applying prodrug and PROTAC technologies collectively. This approach can be applied to improve the stability and delivery of PROTACs. For example, one can modify PROTAC into a prodrug that is more soluble and less prone to degradation. That is why, developing PROTAC prodrugs that are activated only in the desired tissues or cells provides for rather selective protein degradation without side effects. It can be highly effective especially in oncolytics, where this approach can minimise systemic side-effects. Therefore, applying prodrug strategies to PROTACs can improve their pharmacokinetics profile that will allow them to exhibit adequate bioavailability and prolonged efficiency in the target tissue.

Miscellaneous approaches

Conjugates were synthesized by Banik et al. to bind to target proteins' extracellular domains as well as cell surface lysosome targeting receptors. An antibody is linked to agonist glycopeptide ligands for the cation-independent mannose-6-phosphate receptor (CI-M6PR) in these lysosomes targeting chimaeras ("LYTACs"). A CRISPRi knockdown screen, made possible by LYTACs, uncovered the molecular mechanism for cargo internalization mediated by CI-M6PR. As proven, apolipoprotein-E4, EGFR, CD71, and programmed death-ligand 1 (PD-L1) are efficiently degraded by LYTACs. LYTACs provide a modular approach to targeting secreted and membrane proteins for destruction in therapeutic and fundamental research settings [15].

Challenges to PROTACs

Research institutions and pharmaceutical companies alike are keenly interested in PROTAC because of its status as a cutting-edge technology. Like any new technology, PROTAC has potential and obstacles as it develops. Prospects for PROTAC, both positive and negative, will aid in the study and creation of tailored protein-degrading medications. Despite PROTAC's distinct benefits over competing drug development paradigms, it has its share of drawbacks, which pose severe problems Like Resistance, Off target, and Target selection [43]. Because of their unique designs, PROTACs often exhibit poor pharmacokinetic performance and high molecular weight. Despite several reports of PROTAC activity in vivo and their current clinical investigation owing to their unique structures, these compounds often have a significant molecular weight and perform poorly in pharmacokinetic and druggability studies [32]. The potential toxicity and side effects of PROTACs raise significant concerns regarding their clinical application. Unlike traditional inhibitors that only suppress activity without affecting protein expression, PROTACs trigger targeted protein degradation, potentially preventing drug resistance and possibly leading to higher irreversible toxicity [145]. On the flip side, the unintended impact of PROTACs on normal cells or organs could significantly affect medical care. Therefore, it is essential to monitor and assess the toxicological effects of PROTACs rigorously, just as we do for clinical investigations and in the later stages of drug development, and further reduce both on-target and off-target toxicities associated with PROTACs. Generally, the molecular weight of PROTACs falls somewhere between 700 and 1200 Da. Also, compared to standard small-molecule antivirals, PROTACs have more hydrogen bond donors and a more extensive polar region on their surface. These factors can influence the permeability of PROTAC cells for oral administration [146]. Because antiviral PROTACs have to pass across the host cell or even the nuclear membrane to have a biological impact, transmembrane permeability is a potential disadvantage.

Moreover, due to their poor bioavailability and weak pharmacokinetic characteristics, PROTACs do not follow Lipinski's conventional rule of five [147,148]. In clinical treatment, greater dosages of oral

PROTAC medicines are required owing to low oral bioavailability resulting from poor permeability and solubility. This could lead to an increase in the risk of side effects and manufacturing costs. In order to better understand the druggability of antiviral PROTACs, it is essential to create credible and systematic guidelines for optimizing their physicochemical attributes and oral bioavailability.

Conclusion and perspectives

In tumors, immunological illnesses, neurological diseases, cardiovascular diseases and in viral infections the therapeutic potential of PROTAC was explained through the 20 years of PROTAC technology, where some molecules already have come close to clinical stage. Among the new insights there is the fact that PROTACs appear to be sensitive to drug-resistance targets. This method can address the problem of the existing drugs which the current therapies may be encountering such as drug resistance. PROTACs degrade the complete target protein, which affects protein function, which maybe enzymatic or otherwise. The second is 'undruggable targets' as a potential approach to PROTACs. Most researchers agree that greater than 80 percent of proteins in human cells, where drug targets can reside, do not have active sites of binding enzymes or receptors, a significant problem for most small-molecule drugs or large-molecule antibodies. Despite their discovery as enzyme-tagging proteins, distinct functions of PROTACs are possible. Interference with enzymes is known in pharmacology as the traditional type of small-molecule drugs' action. It indicates that PROTACs can regulate not only proteases and other enzymes but also other non-enzyme processes and increase the "drug-target" interface. Several of the issues elicited by classical small molecular inhibitors can, however, be worked around. From this perspective, nanotechnology presents an innovative route to optimise the delivery, performance, and selectivity of PROTACs. The augmentation of PROTACs with nanotechnology can address some of the challenges, which includes solubility, stability and bioavailability can therefore open up more opportunities of therapeutic strategies. One of the advantages of using nanoparticle-based delivery systems is that PROTACs can be included in those nanoparticles to shield it from breakdown in the gastrointestinal tract or the bloodstream. This encapsulation increases the solubility and the stability of PROTACs so that a greater amount of the reagent gets to the target site. They are capable to improve the BBB permeability thus helping in delivery of PROTACs to the affected areas in the body such as the brain in the case of neurodegenerative diseases. Using engineered NPs these barriers can be crossed and PROTACs can be delivered to the central nervous system thereby moving recreational proteins that are linked with neurodegenerative processes. PROTACs carry the potential of future combinations with nanotechnology as ongoing studies include work on better nanoparticle design, better targeting, and far better safety assessments of the delivery systems used. Future evolution of nanotechnology will escalate to the generation of advanced NPs, which would be ligand for PROTACs besides other drugs enhancing the treatment of diseases. Moreover, PROTAC prodrugs do not exist yet in the real-world scenario, and the preliminary findings suggest their effectiveness. For instance, the PROTAC versions of prodrugs can be developed based on the activation by the enzymes that exist in cancer cells, thus providing the direct degradation of oncogenic proteins. Combined application of prodrug and PROTAC technologies seems to have enormous potential to create the new generation of therapeutics with enhanced activity, reduced toxicity and targeting.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

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