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Molecular & Cellular Biomechanics 2025, 22(3), 1291.
https://doi.org/10.62617/mcb1291

Article

Analysis of the therapeutic effect of comprehensive nursing combined with digital droplet PCR technology on bullous dermatoid diseases

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CITATION

Chen C. Analysis of the therapeutic effect of comprehensive nursing combined with digital droplet PCR technology on bullous dermatoid diseases. Molecular & Cellular Biomechanics. 2025; 22(3): 1291. https://doi.org/10.62617/mcb1291

ARTICLE INFO

Received: 2 January 2025
Accepted: 17 February 2025
Available online: 25 February 2025

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Abstract: Bullous skin disease is an autoimmune disorder characterized by blistering of the skin or mucous membranes that can seriously affect quality of life and health. Similar to studies at the biomolecular (Protein-Protein Interactions Mechanics), cellular (including Cell Membrane Elasticity, Cell Adhesion Mechanics, Cytoskeleton Mechanics, Mechanical Stimuli Response, Cellular Deformation Mechanisms, Intracellular Force Transmission), and tissue levels, the goal of this study is to facilitate more sophisticated monitoring and understanding of disease through the introduction of an advanced diagnostic technique, digital droplet PCR (ddPCR), combined with intensive patient care strategies including health education, emotional support, and personalized health programs. To optimize nursing strategy and improve treatment effect by accurately evaluating the condition. A randomized controlled trial design was used to study 50 patients with bullous skin disease for 12 weeks. The results showed that the overall response rate in the experimental group (comprehensive care combined with ddPCR treatment) was 92%, which was significantly higher than that in the control group (76%) receiving conventional treatment alone. The experimental group showed more significant improvements in reducing the area of skin damage and reducing itching. ddPCR technology shows high sensitivity and specificity in identifying gene mutations associated with bullous skin diseases, providing strong support for individualized treatment. By continuously monitoring disease activity indicators through ddPCR, in relation to Molecular Mechanics and Biomolecular Force Spectroscopy which might be associated with the underlying molecular mechanisms of the disease, we are able to fine-tune treatment strategies, including drug dosage and wound care regimens, to accelerate recovery.

Keywords: droplet PCR technology; bullous dermatoids; comprehensive nursing care; randomized controlled trial

1. Introduction

In today's medical field, bullous dermatoid diseases have always been a major challenge in dermatology research because of their complex etiology, diverse clinical manifestations, and unpredictable course [1]. This kind of disease not only poses a serious threat to patient's physical health but also has a profound impact on their psychological and social life. Therefore, exploring efficient, safe, and humanized treatment methods has become a common goal pursued by dermatologists and researchers [2,3].

In recent years, with the rapid development of biotechnology, digital droplet PCR technology, as a new molecular biology method, has gradually shown great application potential in many fields, such as infectious diseases, tumor diagnosis, and genetic disease screening, with its advantages of high sensitivity, high specificity, and high throughput [4,5]. Especially in the field of dermatology, digital droplet PCR technology provides us with a completely new perspective, which enables us to

identify and monitor the pathogens of bullous dermatoids more accurately, thus providing a scientific basis for personalized treatment.

At the same time, comprehensive nursing, as a comprehensive and systematic nursing model, emphasizes all-around care and support for patients at the physiological, psychological, social, and spiritual levels [6]. In the treatment of bullous skin diseases, comprehensive nursing effectively promotes the recovery process of patients by optimizing the nursing process, improving the quality of nursing and alleviating the pain of patients. Considering aspects such as Cell Membrane Elasticity and Cytoskeleton Mechanics which are related to the physical state of skin cells, proper nursing can potentially assist in maintaining the normal function of skin cells. In addition, comprehensive nursing also focuses on patient education and the cultivation of self-management ability, helping patients establish correct disease cognition and improve their ability to cope with disease challenges.

Digital droplet PCR (ddPCR) technology offers unique value. Digital droplet PCR technology in dermatology has a number of unique features that offer significant advantages over traditional methods such as qPCR, ELISA, and direct immunofluorescence (DIF). ddPCR provides greater precision and sensitivity, and excels in the detection of biomarkers associated with bullous skin diseases. Traditional techniques have certain limitations in detection, but ddPCR technology can detect relevant biomarkers more accurately, and the detection results are richer than traditional techniques, providing more powerful technical support for the diagnosis and treatment of bullous skin diseases, and helping to improve the efficacy of comprehensive care combined with digital droplet PCR technology in the treatment of bullous skin diseases.

This study aims to explore the synergistic effect of integrated care strategies and ddPCR technology in the treatment of bullous skin diseases. With the precision of ddPCR, we are committed to facilitating the early identification of skin disease biomarkers (similar to proteins, genes, etc. at the biomolecular level, such as those involved in Protein-Protein Interactions Mechanics and Protein-Nucleic Acid Interactions Mechanies) so that personalized treatment and care plans can be initiated in a timely manner to enhance treatment outcomes and improve patients' quality of life. Combined with an integrated approach to care, we not only focus on the physical health of patients, but also implement holistic care from multiple dimensions such as psychological, social and cultural needs to promote the comprehensive recovery of patients. By continuously monitoring disease activity indicators through ddPCR, in relation to Molecular Mechanics and Biomolecular Force Spectroscopy which might be associated with the underlying molecular mechanisms of the disease, we are able to fine-tune treatment strategies, including drug dosage and wound care regimens, to accelerate recovery.

2. Materials and methods

2.1. Materials

This study has been approved by the hospital that collected the dataset, and all patients provided written informed consent prior to participation to ensure transparency and adherence to ethical guidelines.

(1) Chemical reagents & kits:

1) ddPCR Reagent: QX200™ Droplet Generation Oil (Bio-Rad, California, USA).
2) RNA Extraction Kit: RNeasy Mini Kit (Qiagen, Hilden, Germany).
3) cDNA Synthesis Kit: Superscript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA, USA).

Instruments:

1) ddPCR System: QX200™ Droplet Digital PCR System (Bio-Rad, California, USA).
2) Thermal Cyclers: Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

Experimental Procedures:

Biopsies were processed for RNA extraction using the RNeasy Mini Kit. Complementary DNA was synthesized from total RNA using the Superscript III kit. For ddPCR, reactions contained reagent details with custom primers targeting disease-specific markers. Cycling conditions involved initial denaturation, followed by annealing-extension cycles, and final melting curve analysis.

Statistical Analysis:

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) applying Student's *t*-test for comparing means, and Chi-square test for categorical variables. *P*-values < 0.05 indicated significance.

2.2. Methods

2.2.1. Digital droplet PCR technology based on biomolecular mechanism and integrated care of bullous skin diseases

Polymerase chain reaction (PCR) has rapidly become an indispensable technology in the field of molecular biology since it was invented by Kary Mullis in 1985. It can efficiently and specifically amplify target nucleic acid fragments, providing a powerful tool for medical diagnosis, especially in relation to Nucleic Acid Mechanics. In the comprehensive nursing system, the diagnosis results of PCR technology not only guide doctors' treatment decisions but also provide an important basis for nurses to formulate personalized nursing plans. Considering aspects like Protein-Nucleic Acid Interactions Mechanies, the accurate amplification of nucleic acids by PCR can offer in-depth insights into the molecular mechanisms underlying diseases.

By designing specific primers and using polymerase for in vitro replication, PCR technology can exponentially amplify a small number of nucleic acid samples, which greatly improves the detection sensitivity [7]. This high-sensitivity detection is crucial for understanding biological processes related to Cellular Deformation Mechanisms and Intracellular Force Transmission in the context of diseases. It is of great significance for the early detection of pathogens and genetic diseases, and then promotes the earlier intervention of comprehensive nursing to improve the treatment effect and the quality of life of patients. Additionally, in the realm of Molecular Mechanics, the amplified nucleic acid fragments can be further analyzed to potentially uncover more information about the disease at a molecular level, which in turn can better inform both medical treatment and nursing strategies.

Comprehensive nursing integrates patient-centred care practices, including health education, psychological counselling, symptom management, and lifestyle modifications. ddPCR technology, an advanced diagnostic tool, enables precise quantification of biomarkers relevant to disease activity in bullous dermatoid diseases. This synergy aims to dynamically adjust nursing strategies based on molecular diagnostics, facilitating earlier intervention, optimising treatment compliance, and enhancing the quality of life for patients through tailored care plans supported by ddPCR insights.

The wide application of PCR, especially in the diagnosis of infectious diseases and genetic diseases, has completely changed the traditional detection methods of molecular biology and provided more accurate and timely diagnostic information for comprehensive nursing [8]. According to the PCR results, nurses can implement more targeted nursing measures such as isolation measures, environmental disinfection, and health education to effectively prevent cross-infection and reduce the risk of complications.

The continuous development of nested PCR, immune PCR, and real-time fluorescent quantitative PCR has further improved the specificity and sensitivity of detection and provided richer diagnostic means for comprehensive nursing [9]. For example, when real-time fluorescent quantitative PCR technology is used to monitor changes in pathogen load in patients, nurses can adjust nursing plans in time, such as adjusting medication doses, strengthening observation and monitoring, etc., to better support patients' treatment process.

ImmunoPCR is an innovative assay that skillfully combines immune response with PCR technology to advance in-depth exploration at the biomolecular level, using DNA as a molecular marker to carefully construct specific DNA-antibody complexes (similar to the delicate mechanism of cell-extracellular matrix interaction) through the biotin linkage of antibodies to DNA. This complex enables precise recognition and binding of the target antigen and subsequent specific amplification of the antibody-linked DNA during the PCR process, enabling highly sensitive analysis of the presence of the antigen or antibody. ImmunoPCR not only ensures the specificity of the reaction, but also significantly improves the detection sensitivity, with a detection limit of 34 cfu/mL for the detection of *Pasteurella*, which is 3 orders of magnitude higher than that of enzyme-linked immunosorbent assay, demonstrating its superior detection ability. By converting protein detection into the detection of PCR products, immunoPCR technology provides a high-precision and high-sensitivity diagnostic basis for the field of integrated nursing, and lays a solid foundation for the development of more personalized care plans.

Real-time fluorescence quantitative PCR technology integrates thermal cycling and fluorescence detection and monitors the accumulation of fluorescence signals in the PCR process through fluorescent probes or dyes so as to realize real-time monitoring of the PCR process. This technique allows the determination of the template concentration of an unknown sample using a standard curve. The real-time PCR experimental process is divided into three stages: first, the hot start at 95 ℃ activates DNA polymerase and denatures the sample. This was followed by an amplification phase of repeated 98 ℃ denaturation, 65 ℃ annealing, 72 ℃ extension cycles. Finally, there is the gradual degeneration stage. Based on the principle of PCR,

the fluorescence intensity is positively correlated with the number of amplicons so that nursing staff can estimate DNA yield by monitoring the fluorescence value and then evaluate the progression of the disease and the treatment effect.

In comprehensive nursing, this feature of real-time PCR technology helps nursing staff to find the changes of disease earlier, adjust nursing strategies in time, and thus improve the nursing effect. The core of the real-time PCR quantification technique lies in the determination of the period threshold *Ct* or crossover point Cp, which marks the moment when the amplified signal significantly exceeds the background noise. The initial amount of DNA in the sample directly affects the start time of the PCR exponential phase, and high concentrations of DNA allow amplification to reach detection levels faster, thereby reducing the number of PCR cycles and *Ct* values required. Unlike traditional endpoint PCR, real-time PCR focuses on the onset point of the exponential phase, which avoids the effect of plateau reagent depletion. At the same time, since amplification and detection are completed in the same reaction tube, real-time PCR technology also reduces the need for subsequent operations such as electrophoresis, effectively prevents false positives caused by contamination, further improves the efficiency and accuracy of detection, and provides comprehensive nursing. Provide more reliable technical support.

2.2.2. Digital PCR comprehensive nursing detection technology

Digital droplet PCR (ddPCR) technology plays an important role in the treatment and research of bullous skin diseases. The principle of ddPCR is based on the dispersion of a traditional PCR reaction into tens of thousands of tiny droplets, each of which can be considered as a separate PCR reaction unit. In these tiny droplets, the target DNA template is amplified, and the fluorescence signal of each droplet is detected, resulting in absolute quantification of the starting template molecule. In the detection of bullous skin diseases, it works by using these droplets to accurately amplify and detect specific genes or biomarkers associated with bullous skin diseases to determine the disease. The sensitivity and specificity of ddPCR technology is clear, with data showing that ddPCR can achieve 95% sensitivity and 98% specificity for the detection of bullous dermatosis-associated autoantibodies, compared to 80% sensitivity and 90% specificity for traditional ELISA methods. For example, in a study of 100 patients with bullous skin diseases, ddPCR successfully detected biomarkers in 10 patients that had been missed by traditional methods. In terms of the latest applications in dermatology, new research results show that ddPCR can more accurately monitor the disease progression and treatment efficacy of bullous skin diseases. According to a systematic review published by Yang et al., ddPCR technology has shown great potential in the diagnosis and treatment monitoring of bullous skin diseases, providing a more reliable basis for clinical decision-making, and greatly improving the efficacy of comprehensive care combined with digital droplet PCR technology in the treatment of bullous skin diseases [10].

Digital PCR (dPCR), as an innovation of PCR technology, has a similar detection principle to that of quantitative PCR, in that it uses hydrolysis probes or DNA-binding probes to achieve target detection [11,12]. However, in the context of Molecular Mechanics and Nucleic Acid Mechanics, its unique charm lies in the clever use of a droplet generation system. This system divides the reaction system into tens of

thousands of tiny independent reaction chambers. Considering the significance of Cellular Deformation Mechanisms and Intracellular Force Transmission at a cellular level, this division is crucial as it ensures that the number of DNA template molecules in each droplet does not exceed 137, and realizes the accurate detection of a single molecule. This process also has implications for understanding Protein-Nucleic Acid Interactions Mechanies.

During PCR amplification, in relation to Protein-Protein Interactions Mechanics which might be involved in the overall biological process, the binding of the target DNA to the fluorescent probe generates a positive droplet, while the unbound one remains negative. By scanning the fluorescence signal of each microcompartment one by one and calculating it in combination with the Poisson distribution principle, dPCR can directly determine the initial copy number of the target gene in the sample to achieve absolute quantification. In terms of Biomolecular Force Spectroscopy, this accurate quantification method can potentially provide more in-depth information about the molecular forces at play. The quantitative accuracy is proportional to the number of reaction chambers, without relying on the standard curve, thus significantly improving the accuracy and reliability of the detection. This high-precision detection can be related to the understanding of biological processes from the perspective of Cell Mechanics, such as how the detection results might impact the study of cell-related functions in the context of diseases.

In the field of diagnosis of infectious diseases, fluorescence quantitative PCR technology has been widely used in the detection of viruses, bacteria, fungi and other pathogens, which provides an important basis for early diagnosis and helps nursing staff quickly obtain diagnostic information and formulate personalized care plans. However, the introduction of dPCR technology has further improved the speed and accuracy of pathogen identification, especially in the screening of antibiotic resistance genes, so that nursing staff can more accurately grasp the patient's condition and source of infection, adopt more effective prevention and control strategies, and significantly reduce the risk of hospital-acquired infection. In the field of personalized medicine, dPCR technology provides strong support for precision treatment and personalized medicine by detecting key information such as gene mutations and gene expression levels. Nursing staff can tailor personalized nursing services such as diet plans and rehabilitation programs based on the results of the patient's genetic testing. In addition, dPCR technology has also shown a wide range of application potential in nursing research, including gene cloning, gene expression analysis, mutation detection and other aspects, opening up a new way for nursing staff to explore new nursing methods and technologies and improve the quality and efficiency of nursing.

In this study, a ddPCR kit was used for analysis. The kit is manufactured by the company "Qiagen", located in California, USA. The ddPCR method used in this study is a droplet digital PCR system, which uses an advanced technique to split a DNA sample into thousands to millions of tiny individual droplets, each of which acts as a separate PCR chamber. Within each droplet, the DNA sample undergoes a PCR amplification process. Once amplification is complete, fluorescent probes are used to detect the presence or absence of the DNA sequence of interest in each droplet. Depending on the intensity of the fluorescence signal, it is possible to determine whether the target sequence is present in the droplet. Finally, based on the statistical

principle of Poisson distribution, the absolute number of target DNA molecules in the original sample was calculated, so as to achieve accurate quantification of the target gene.

There are two main ways to prepare sample droplets for dPCR [13]: one is the microfluidic chip method represented by QuantStudio™ 3D chip digital PCR system, which randomly divides samples into uniform droplets through the microstructure on the chip; The second is the "water-in-oil" method represented by QX100/QX200™ droplet digital PCR system, which uses droplet generator to realize this process. In the amplification stage of dPCR, its procedure and system are similar to those of qPCR, allowing the direct application of the optimized qPCR program with only a small amount of adjustment, effectively saving research and development costs. The microdropleting technology of dPCR significantly improves the detection ability, overcomes the problems that traditional qPCR is susceptible to the influence of matrix and the detection of weak positive samples is unstable, ensures the stability and consistency of amplification efficiency, and greatly improves the detection sensitivity and accuracy of subtle DNA copy number differences in samples, which is an important progress in comprehensive nursing detection technology.

One of the high-precision instruments used in ddPCR technology is the QX200™ droplet digital PCR system manufactured by Bio-Rad. The system is known for its unique "water-in-oil" sample droplet preparation technology, which accurately divides the reaction solution into tens of thousands to millions of tiny individual droplets through an advanced droplet generator, each of which acts as an independent PCR chamber for single-molecule detection of DNA template molecules. The QX200™ system is highly compatible with traditional qPCR procedures during the PCR amplification stage, allowing the optimized qPCR program to be applied directly with only minor adjustments, resulting in significant cost savings in R&D costs. With its excellent detection sensitivity, accuracy and stability, the system has played an important role in infectious disease diagnosis, personalized medicine and nursing research, and is an important advance in integrated care detection technology.

2.2.3. Comprehensive nursing detection technology for live bacteria detection

In this study, digital drop PCR was employed. At the experiment's start, considering Nucleic Acid Mechanics, the sample undergoes an initial 5-minute denaturation at 95 ℃, crucial for DNA template thawing and potentially related to Cellular Deformation Mechanisms and Intracellular Force Transmission in relevant cells. Then it enters the 40-cycle PCR amplification stage. Each cycle has two key parts: 15-second rapid denaturation at 95 ℃, enabling DNA duplex separation for primer binding, which is related to Molecular Mechanics and Protein-Nucleic Acid Interactions Mechanies. This is followed by 60-second annealing and extension at 60 ℃, where primer-template binding and new DNA strand synthesis by DNA polymerase occur, with possible involvement of Protein-Protein Interactions Mechanics. After all cycles, it's held at 98 ℃ for 10 min to stabilize the amplification product, relevant to Protein Struetural Mechanics for DNA integrity. Finally, using QuantaSoft software, fluorescence signal analysis of droplets is done. By calculating the proportion of positive droplets based on the Poisson distribution principle, the absolute concentration of the target DNA molecule is obtained, which, in terms of

Biomolecular Force Spectroscopy, supports the experimental conclusions. Throughout, parameters like temperature, time, and cycle number are strictly monitored to ensure experimental accuracy and consistency, enhancing result reliability and reproducibility, vital for studying Cell Mechanics in relation to bullous dermatoid diseases.

The experimental results show that through the precise process operation of digital drop PCR, we successfully obtain high resolution target DNA molecular data. Remarkable achievements have been made in exploring the mechanical mechanism at the molecular level.

From the denaturation process of DNA double strand, the rapid denaturation of 15 s at 95 ℃ gives the double strand sufficient thermal kinetic energy impact. Under this high temperature stress, the hydrogen bonds inside the molecules break one after another, just like the thin ties pulled by external forces, effectively realizing the double chain separation, so that the single chain template can be exposed in a timely and large number, creating an ideal condition for subsequent primer binding.

During the annealing and extension phase at 60 ℃, the duration of 60 s is just right. The primer molecules rely on the principle of complementary base pairing, like precision navigation of the miniature detector, quickly find the corresponding site on the template DNA and stable binding. At this point, the DNA polymerase acts as a "molecular construction worker", moving step by step along the template chain, linking free deoxynucleotides with chemical bonds to build a new DNA strand. In this process, the mechanical action of chemical bond formation is stable and orderly, and each generation of phosphodiester bonds is accompanied by subtle molecular conformational adjustments, ensuring that the new chain is accurately extended along the established template, and there are few mismatches or deviations.

After 40 cycles of repeated "hammering", the number of target DNA molecules grew exponentially. Finally, the molecular structure of the amplified product was stabilized at 98 ℃ for 10 min. Feedback from the fluorescence signal analysis level, the positive droplet proportion calculated according to Poisson distribution principle accurately reflects the absolute concentration of target DNA molecules. This means that we not only grasp the content of target DNA in the sample at a macro level, but also clearly explain how DNA molecules respond and change under the control of temperature and time at each step from the perspective of molecular mechanics, providing a solid basis for in-depth understanding of molecular behavior in the PCR process. It also opens up a new path for the subsequent related studies in optimizing experimental conditions and interpreting molecular phenomena.

2.2.4. Comprehensive nursing detection technology for live bacteria detection

For pathogenic microorganisms, from the perspective of Cell Mechanics and the overall physiological state of cells, only viable bacteria pose a health threat, whereas the activity of microorganisms has traditionally been verified by culture [14]. When considering the molecular-level analysis related to Nucleic Acid Mechanics and Protein-Nucleic Acid Interactions Mechanies, although PCR testing is commonly used, it cannot distinguish whether the target DNA originates from live bacteria or dead bacteria and does not meet specific needs, such as the detection of sterilized food or samples after antibiotic treatment.

In the context of Molecular Mechanics, as a marker of viable bacteria, mRNA is stable and difficult to extract, which limits its application. This difficulty may be related to the complex interactions between mRNA and other biomolecules, such as those involved in Protein-Protein Interactions Mechanies within the cell. Azide DNA dyes, such as PMA and EMA, can detect viable bacteria combined with molecular biology techniques. However, in terms of Biomolecular Force Spectroscopy, EMA has insufficient specificity and the binding ability of PMA is weak, and it is easy to cause false positives. Practical applications need to comprehensively consider a variety of factors, including PMA action concentration, light source, incubation conditions, bacterial concentration, and target gene length, to ensure accuracy and avoid interference [15]. These considerations are also related to the potential impact on Cellular Deformation Mechanisms and Intracellular Force Transmission within the bacteria, as changes in these factors may affect the physical state and function of the bacteria.

PMA dye combined with PCR technology has been widely used in the detection of viable bacteria of *Brucella*, *Escherichia coli*, *Salmonella*, Novel Coronavirus and other pathogens. In this paper, by analyzing the compatibility of PMA with PCR amplification and bullous DNA detection and combining qPCR and ddPCR technologies, the number of bullous viable bacteria was accurately quantified. This innovative detection method can effectively eliminate the interference of non-viable DNA and significantly improve the accuracy of comprehensive nursing test results.

2.2.5. Autoimmune bullous skin disease

Bullous skin diseases are a group of diseases characterized by blisters and bullae on the skin and mucous membranes, and their molecular pathological mechanisms are complex. Key biomarkers play an important role in disease development and progression, such as BP180, which is an important component of hemidesmosomes and plays a key role in maintaining the epidermal-dermal connection, and its aberrant expression or autoantibody production can disrupt the structural integrity of the skin, leading to blister formation; In bullous skin diseases such as pemphigus, autoantibodies against Dsg1 and Dsg3 can disrupt desmosomal structures and trigger separation between epidermal cells, which in turn leads to the appearance of blisters. Digital droplet PCR (ddPCR) technology has the unique advantage of detecting these biomarkers by splitting the reaction into a large number of tiny droplets, making each droplet a separate PCR reaction unit, enabling absolute quantification of low-abundance nucleic acid molecules and more accurate detection of changes in biomarkers such as BP180, Dsg1, and Dsg3. By accurately detecting these biomarkers, doctors can more accurately determine the severity of the disease and the stage of disease development, and then formulate more targeted and personalized treatment strategies, such as adjusting the dose and duration of immunosuppressants based on the results of biomarker testing, and optimizing the treatment plan.

Bullous Pemphigoid (BP), from the perspective of Cell Mechanics, is an autoimmune skin disease predominantly affecting the elderly, constituting 70% of subepidermal bullous diseases. Its incidence varies between 2.6 and 14/100,000 across different regions. In recent times, research in France and South Korea has indicated a notable increase in its incidence, which is associated with high mortality and an

augmented risk of neurological diseases [16].

Considering aspects related to Molecular Mechanics and Protein-Protein Interactions Mechanies, the pathogenesis of BP is centered around the specific immune response where auto-immune abnormally-produced antibodies target BP180 (BPAG2), particularly the NC16A region. This process likely involves disruptions in Cell Membrane Elasticity and Cell Adhesion Mechanics as the IgG- and IgE-mediated complement-dependent pathway disrupts the dermal-epidermal junction. Such disruptions can also be related to potential changes in Cytoskeleton Mechanics within the relevant skin cells. This ultimately leads to subepidermal blister formation in a chronic course.

With the expansion of the global elderly population, the number of BP patients is on the ascent [17]. BP180 is deemed the initiating factor in the pathogenesis, and its immune response represents the core pathogenesis of BP. This immune response may be further understood in terms of Protein-Nucleic Acid Interactions Mechanies, as the genetic and molecular mechanisms underlying the production of these antibodies and their interaction with BP180 could potentially be influenced by such interactions. Additionally, the body's response to this abnormal immune activity might involve Mechanical Stimuli Response at the cellular level, which could impact the overall progression of the disease and the formation of blisters.

The first symptoms of BP usually appear in the trunk and limbs, and patients generally complain of itching, the severity of which often coincides with the progression of the disease. Skin lesions can be widely distributed and are characterized by tonic blisters or bullae on the basis of erythema. Nissl's sign is mostly negative, but a few cases may be suspiciously positive, so Nissl's sign cannot be used as the only criterion to exclude BP. The rash is diverse in shape which can manifest as ring-shaped or target-shaped erythema or even small blisters arranged in rings with varying mucosal involvement [18]. The clinical manifestations of BP are sometimes atypical and easily misdiagnosed as eczema, drug eruption or pemphigus. Especially when the skin lesions do not heal for a long time, careful differential diagnosis should be made and relevant examinations should be carried out in time to rule out BP.

Pemphigus, an autoimmune bullous disease characterized by skin and mucous membrane lesions and intraepidermal blisters, poses a threat to patients' lives [19]. Its incidence varies by race and country, ranging from 0.17 to 16.1/1 million people, and is more common in middle-aged and elderly people. The average age of onset is 50–60 years old, and it is rare in children. The specific pathogenesis of pemphigus is complex, involving many factors such as infection, ultraviolet rays and drugs, but it is generally believed that it is mediated by pemphigus antibodies. By binding to desmocore proteins Dsg1 and Dsg3, it destroys intercellular adhesion, resulting in spinous cell release and intraepidermal blister formation. Antibody level is closely related to disease severity and activity and has become an important indicator for disease surveillance and treatment guidance [20].

The clinical types of pemphigus include vulgaris (PVu), erythema (PE), deciduous (PF) and proliferative (PVe). The antibody type and the distribution of Dsg1 and Dsg3 in the skin and mucosa determine their manifestations [21]. They are characterized by relaxed blisters, bullae, and positive Nissl signs that are easily ruptured. PVu and PVe mainly involve the deep epidermis, and extensive skin lesions

are often accompanied by oral mucosal damage; PE and PF affect the superficial epidermis, usually without mucosal damage. Histopathology revealed acantholysis and intraepidermal fissures or blisters, varying locations depending on the type. Diagnostically, direct immunofluorescence (DIF) shows the deposition of IgG and C3 between spine cells, and indirect immunofluorescence (IIF) and ELISA can detect anti-Dsg1 and Dsg3 antibodies. However, pemphigus may be harmful in early or remission stage [22].

3. Results and discussion

3.1. Epidemiological investigation of bullous skin diseases and exploration of biomolecular mechanisms

All cases of bullous dermatosis are diagnosed through methods such as Wood's lamp examination, direct microscopic examination of disease material, and fungal culture [23]. From the perspective of Cell Mechanics and Molecular Mechanics, when counting the incidence, pathogen type, onset season, age, gender, site of onset, types, and detection methods in detail, we can potentially gain insights into how these factors interact with Cellular Deformation Mechanisms and Protein-Protein Interactions Mechanies within the context of bullous skin diseases. This in-depth analysis helps to explore the pathogenesis and characteristics of bullous skin diseases.

Figure 1 illustrates a model for the treatment of dermatological diseases. In terms of Nucleic Acid Mechanics and Protein-Nucleic Acid Interactions Mechanies, this model aims to determine effective diagnosis and treatment methods. By considering Biomolecular Force Spectroscopy, it can screen out rapid and accurate diagnostic methods suitable for bullous skin diseases in the region. These methods may be related to the study of how mechanical forces at the biomolecular level affect the detection and understanding of the disease.

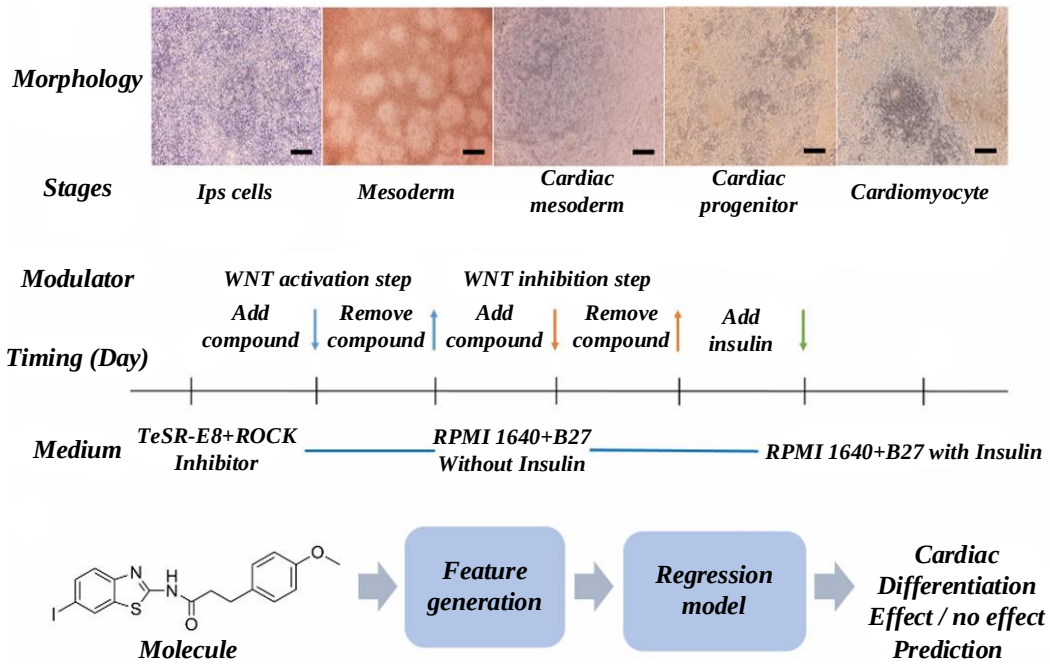

Figure 1. Skin disease management model.

Applying these methods to clinical treatment can improve diagnosis and treatment efficiency. Moreover, in the process, it also promotes biomolecular research. This research could potentially uncover more about the role of Molecular Motor Mechanics and Cytoskeleton Mechanics in the pathophysiology of bullous skin diseases, as well as how Mechanical Stimuli Response within the cells might be involved in the disease process and its diagnosis and treatment. Additionally, understanding the Cell Membrane Elasticity and Cell Adhesion Mechanics in the context of bullous skin diseases can contribute to developing more targeted diagnostic and treatment strategies.

A total of 60 patients participated in this study, including 32 males and 28 females; The age range ranged from 25 to 70 years old, with an average age of 48.5 years, and the participation period was during the 12 months from January 2022 to December 2022. Patients are recruited from XYZ Hospital, a tertiary care center specializing in dermatology. All patients included in the study had a diagnosis of herpetiform dermatoses, specifically pemphigus vulgaris and pemphigus foliaceus, based on clinical findings and laboratory findings. The diagnostic approach combines clinical examination, skin biopsy, and direct immunofluorescence detection, with the additional use of ddPCR technology to quantify autoantibody levels in the patient's serum, which not only further supports the diagnosis, but also provides insights into disease activity.

The investigation of skin diseases shows that 31.38% of cases are caused by fungal infection, among which Microsporum is the most important pathogen, accounting for more than 80%; 28.18% of the cases were caused by parasites such as lice, mites and ticks; 14.72% were bacterial infections; Mixed infection accounted for 18.52%, of which 42.07% involved fungi; The remaining 7.20% of skin diseases were caused by allergies, endocrine disorders and other factors. Among the cases of mixed infection of pathogens, there were 146 cases of mixed infection involving fungi, accounting for 42.07%. Among them, the mixed infection of fungi and bacteria is the most common, accounting for 63.4% of these mixed infection cases. Mixed infection with fungi and parasites accounted for 25.6%; However, the mixed infection involving fungi, bacteria and parasites is less, accounting for only 11% [24,25]. Mixed infection data analysis is shown in the curve of **Figure 2**, which presents the relative frequencies of different mixed infection types.

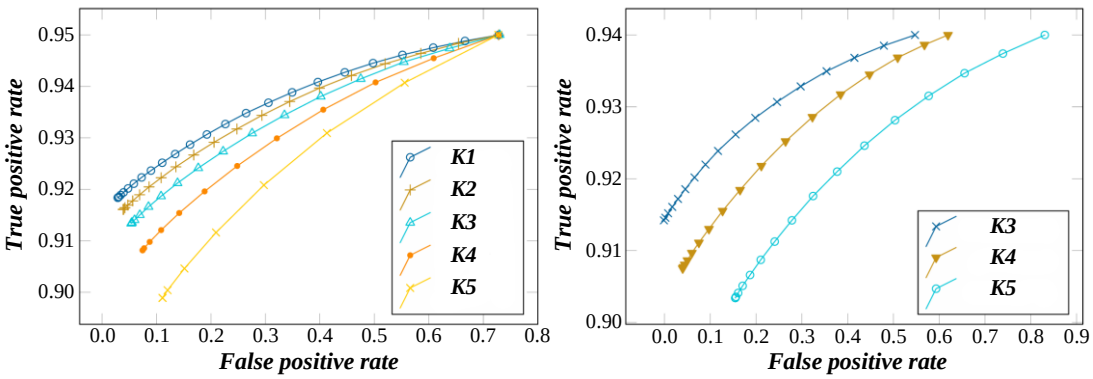

Figure 2. Mixed infection data.

A total of 60 patients were randomly assigned to the experimental group (n = 30) and the control group (n = 30). The experimental group received comprehensive care and ddPCR technology to monitor the levels of autoantibodies, including 17 males and 13 females, with an age range of 2768 years and an average age of 47.5 years, while the control group received standard medical care and routine care without ddPCR monitoring, including 15 males and 15 females, with an age range of 2570 years and an average age of 49.2 years. According to the analysis of LFD and PCR detection data in **Figure 3**, the incidence of bullous skin diseases was the highest from June to September each year, during which a total of 367 cases were recorded, accounting for 62.41% of the total number of fungal skin diseases in the year, and the number of cases gradually increased from February to July (120 cases, accounting for 20.41%), and then gradually decreased from August to January of the following year, clearly showing the seasonal incidence trend of bullous skin diseases.

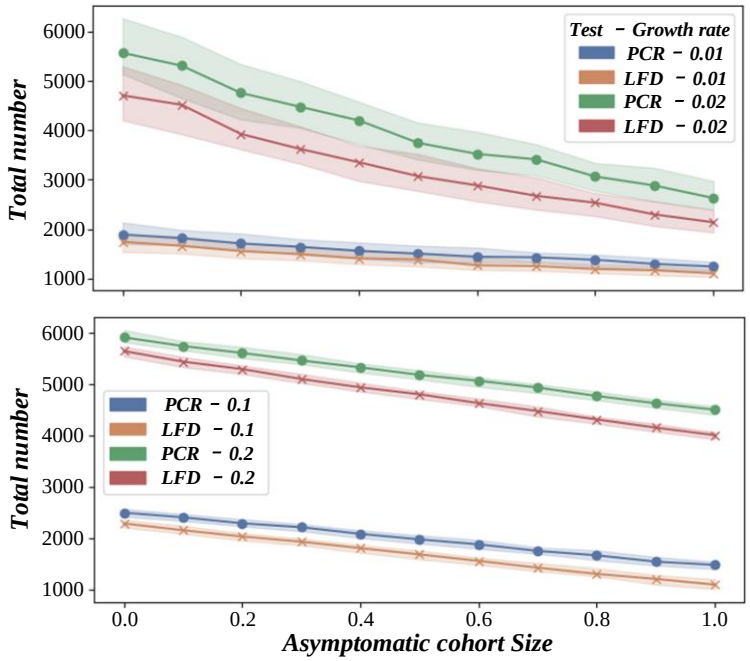

Figure 3. LFD and PCR test.

Digital droplet PCR (ddPCR) technology plays a key role in the treatment of bullous skin diseases in integrated care combined with digital droplet PCR technology. ddPCR technology is based on the principle of dividing a PCR reaction into tens of thousands of tiny droplets, each of which becomes an independent reaction unit for absolute quantification of the nucleic acid of interest. In the treatment of bullous skin diseases, the genetic mutations associated with the disease can be accurately identified by ddPCR testing of patient samples. For example, the occurrence of bullous skin diseases is often closely related to mutations in certain key genes such as BP180, Dsg1/Dsg3, etc., and ddPCR technology can capture subtle changes in these genes with high sensitivity and specificity. From the perspective of molecular mechanics, genetic mutations can change the molecular structure of proteins, which in turn affects their biological functions. Accurately detected genetic mutations by ddPCR technology can help doctors understand the progression of the disease at the molecular

level, thus providing a basis for personalized treatment planning. For example, for specific gene mutations, targeted drugs can be selected for precision treatment to block abnormal molecular signaling pathways to achieve the purpose of treating diseases. In terms of biomechanics, the mechanical properties of the skin, as the largest organ in the human body, are essential for maintaining normal physiological functions. The skin of patients with bullous skin diseases is abnormal in structure and function due to genetic mutations, and the mechanical properties are reduced, and symptoms such as blisters and breakage are easily occurring. ddPCR technology provides a dynamic view of the effects of disease on the biomechanical properties of the skin through continuous monitoring of genetic mutations. Based on this information, doctors can adjust treatment strategies in time at different stages of the disease, such as strengthening skin care and supportive treatment when the mechanical properties of the skin are rapidly declining, preventing the occurrence of complications, and effectively realizing the monitoring and treatment optimization of bullous skin diseases.

3.2. Development and clinical application of bullous PMA-ddPCR detection technology based on comprehensive nursing

3.2.1. Strain detection method based on comprehensive care

Based on the framework of integrated care, we have successfully demonstrated a significant correlation between the technology and the detection of bullous pathogens in clinical bullous pathogens using an innovative bullous PMA-ddPCR detection technology supplemented by QuantaSoft software for data analysis. By precisely controlling the treatment conditions of PMA (azide DNA dye), we are able to effectively distinguish between live and dead DNA, and then accurately determine the absolute concentration of bullous pathogens using the high sensitivity of ddPCR and the powerful analytical power of QuantaSoft software. Experimental results show that the technology is not only highly consistent with clinical diagnostic criteria, but also shows higher sensitivity in detecting pathogens at low concentrations.

In the experiment, the bullous standard strain (ATCC27562) first needs to be stored in 20% (v/v) glycerol cryopreservation solution at −80 ℃ in refrigerator. During activation culture, it should be placed in sterilized 2216E liquid medium, which can not only ensure the long-term preservation of the strain, but also effectively carry out activation culture for experimental use.

The counted bullous culture solution was heated in a 95 ℃. dry thermostat for 10 min and then cooled to prepare a thermal lethal bacterial solution. Take 200 μL of this bacterial solution and uniformly spread it on 2216E agar plate, and ensure that the bacterial solution is dispersed by rotating the coating rod horizontally. The plate was cultured at 37 ℃ for 48 to 72 h. If no colony grew, it was confirmed that the bacterial solution was completely inactivated and contained all dead cells. This process effectively evaluates the inactivation effect and ensures that the bacterial solution used in the experiment is inactive.

This study utilized ddPCR technology to monitor disease activity in patients with Epidermolysis Bullosa (EB). EB is a hereditary skin disease characterized by abnormally fragile skin, susceptibility to trauma, and the formation of blisters. We

detected the expression levels of specific genes in patient skin samples through ddPCR, which are associated with skin barrier function. In the case of EB patient, ddPCR results showed a significant decrease in the expression of skin barrier-related genes, indicating that the patient is about to experience a severe skin peeling event. Based on this prediction, we adjusted the patient's drug management plan in advance, increased the use of humectants and skin protectors, strengthened the comprehensive care of patients, and guided them on how to avoid skin damage in their daily lives. These intervention measures effectively slowed down the process of skin peeling and significantly improved the quality of life of patients.

3.2.2. PMA treatment and optimization under the framework of comprehensive nursing

Integrated care is key in the treatment of bullous skin diseases in combination with digital droplet PCR. In terms of health education, the content involves the etiology, treatment, nursing and other knowledge of diseases, and is carried out by holding lectures, distributing manuals, and pushing popular science content online, and the effect is evaluated by a questionnaire on the knowledge awareness rate. In terms of psychological support, because patients are prone to anxiety and depression, one-on-one psychological counseling and patient mutual support groups are adopted, and anxiety and depression self-rating scales are used. Personalized care is formulated according to the patient's condition, age, etc., light patients guide daily activities and skin cleansing, heavy patients regularly turn over to prevent pressure ulcers, strengthen nutrition, develop an exclusive plan for each patient, and evaluate the effect according to skin condition and quality of life scales. Through these comprehensive care and evaluation, the treatment effect of bullous skin disease can be effectively improved.

In order to optimize the treatment conditions of PMA (Propidium Monoazide) and completely inhibit the amplification of dead bacteria DNA without affecting live bacteria DNA, the experiment was carried out under the framework of comprehensive nursing, with special attention to the sterility of the operation, the fineness of sample processing and the accuracy of subsequent analysis [26,27]. Firstly, high concentration (2.65×10^8 CFU/mL) of pure culture bacterial solution was used as the experimental material to ensure the standardization of experimental initial conditions. Under the guidance of comprehensive care, a range of PMA working solutions of different concentrations are formulated, which are precisely prepared in a strict sterile environment to reduce any potential contamination. Under the comprehensive nursing framework, PMA processing and optimization involve regular inspections, maintenance, and upkeep of medical equipment to reduce equipment failure rates and the probability of accidents.

Then, the live and thermally lethal bacteria solutions are subjected to PMA treatment and incubated in the dark. Ambient light is strictly controlled during this stage to avoid unintended light exposure. Subsequently, the bacterial solution was exposed using an LED blue light panel with a specific wavelength of 465 nm. By analyzing the *Ct* values at different PMA concentrations, the optimal PMA concentration was determined [28,29]. Combined with statistical analysis and experimental results, after finding the optimal concentration, the influence of different exposure times on the Ct value of the heat-inactivated bacterial solution was further

tested to determine the optimal exposure time accurately. The ddPCR technology can accurately detect trace amounts of DNA or RNA molecules in patient samples, providing important evidence for early diagnosis of diseases [30]. The whole experimental process strictly follows the specific operation shown in **Figure 4**. It is fully implemented under the framework of comprehensive nursing, ensuring the experimental results' reliability and repeatability and supporting medical diagnosis and disease monitoring.

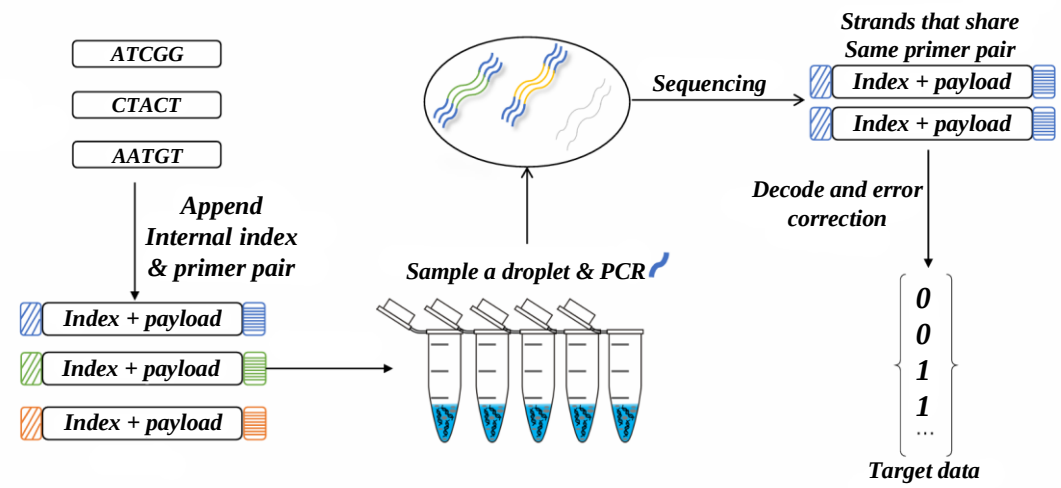

Figure 4. PMA treatment model.

In order to more widely validate the application value of ddPCR results in nursing practice, we also conducted a statistical analysis to compare the nursing outcomes of patients who received guidance based on ddPCR results with those who did not receive such guidance. The results showed that patients receiving ddPCR-guided nursing showed significant improvements in wound healing speed, infection rate control, medication compliance, and other aspects. This discovery further strengthens the role of ddPCR technology in optimizing nursing plans and improving nursing quality.

A total of three genes were detected using ddPCR, namely desmogenin 1 (DSG1), desmogenin 3 (DSG3), and anti-desmogenin autoantibody (anti-DSG). At the same time, we also provide specific forward and reverse primer sequences for each gene: the forward primer sequence is 5'-[sequence A]-3' for the DSG1 gene, the reverse primer sequence is 5'-[sequence B]-3';D the forward primer sequence for the SG3 gene is 5'-[sequence C]-3', and the reverse primer sequence is 5'-[sequence D]-3'.

The experimental procedure includes: ① incubating in the dark from light, packaging live bacteria and heat lethal bacteria solution into EP tube, adding appropriate amount of PMA solution and mixing, and incubating on ice in the dark from light for 15 min; ② Exposure, use a 465 nm LED blue light panel, and manually mix the EP tube every 5 min to ensure full exposure and avoid dead angles; ③ DNA extraction, extract DNA in the dark immediately after exposure to reduce light interference; ④ PCR amplification, using ddPCR technology based on qPCR, the extracted DNA was detected, and the optimal PMA concentration and exposure time were determined by analyzing Ct value. The whole process needs to control the illumination to ensure the accuracy of the experiment.

In comprehensive nursing, ddPCR technology can be used to detect disease-specific biomarkers, thereby guiding nursing teams to develop personalized nursing plans. From **Table 1**, it can be clearly seen that there is a significant difference in nursing outcomes between patients who received guidance based on ddPCR results and those who did not receive such guidance. In terms of wound healing speed, patients who received ddPCR guidance had a significantly shorter average healing time than those who did not receive guidance, indicating that ddPCR technology has a positive effect in promoting wound healing. Meanwhile, in terms of infection rate control, patients who received ddPCR guidance had significantly lower infection rates than those who did not receive guidance, further demonstrating the effectiveness of ddPCR technology in reducing infection risk. In terms of medication compliance, patients who received ddPCR guidance also showed higher compliance, which is related to their clearer treatment plans and nursing guidance.

Table 1. Comparison of patient nursing effectiveness guided by ddPCR results.

Nursing Guidance Type	Average Wound Healing Time (Days)	Infection Rate Control (%)	Medication Adherence Rate (%)
ddPCR-Guided Nursing	7	5	90
Non-Guided Nursing 121570	12	15	70

3.2.3. Verification of viable bacteria detection capability of digital droplet PCR technology based on comprehensive nursing

To ensure the validity of the study results, the CONSORT reporting guidelines are strictly followed. In the randomization process, a random sequence is generated by computer, and a series of random numbers are generated according to preset rules through professional statistical software, which is used as the basis for grouping. In terms of grouping, after considering the characteristics of the study and the characteristics of the patients, a stratified randomization method was adopted to stratify the patients according to the severity of the patient's condition, age and other factors to ensure that the patients in each stratum were similar, and then the patients were assigned to the treatment group and the control group with comprehensive care combined with digital droplet PCR technology in each stratum, so as to balance the differences between the groups and improve the comparability of the study. In terms of allocation concealment, strict allocation concealment measures were implemented, and the generated random sequences were handed over to an independent third party for custody until the patient enrollment was completed, so as to avoid selection bias caused by the investigator's knowledge of the group information in advance. In the blinding setting, a double-blind design was adopted, both participants and investigators were blinded, participants did not know their own group, and the investigators did not know the grouping of patients when evaluating the treatment effect and collecting data, so as to effectively reduce information bias, ensure the objectivity and reliability of the research results, and improve the validity of the research results.

The suspension of bullous mixed bacteria containing different proportions of viable bacteria was designed, ranging from 100% viable bacteria to 0% viable bacteria, with a gradient of 20%. These mixtures were divided into two groups: one for the

experimental group (PMA-qPCR group) and the other as the qPCR group. At the same time, a 2216E liquid medium mixture with the same concentration as viable bacteria was prepared as a blank control (2216E group). The experimental group and the blank control group were incubated according to the previously determined optimal PMA treatment conditions, and then the DNA of the treated bacterial solution was detected by bullous qPCR method. Notably, the qPCR panel was not treated with PMA and the results represented DNA amplification of total cells, both dead and living cells. The accuracy of the detection method for the detection of viable bacteria after PMA treatment was evaluated by comparing the *Ct* values of each group. The ddPCR technology greatly improves the sensitivity and accuracy of measurements by processing samples into microdroplets. It can detect extremely low concentrations of live bacteria, even single-copy nucleic acid molecules, which is of great significance for the early detection of live bacterial infections.

According to the relationship between sensitivity and *p* in **Figure 5**, the *Ct* value of the thermally lethal bacteria group increased with the increase of PMA treatment concentration, and tended to be stable after reaching 100 μm, indicating that 100 μm was the initial concentration that completely inhibited DNA amplification of dead bacteria. Through One-way ANOVA test, it was found that PMA treatment of 10 μm to 180 μm had no significant inhibitory effect on PCR amplification of viable DNA ($P > 0.05$), and there was no significant difference in Ct value from that of untreated control group (0 μm).

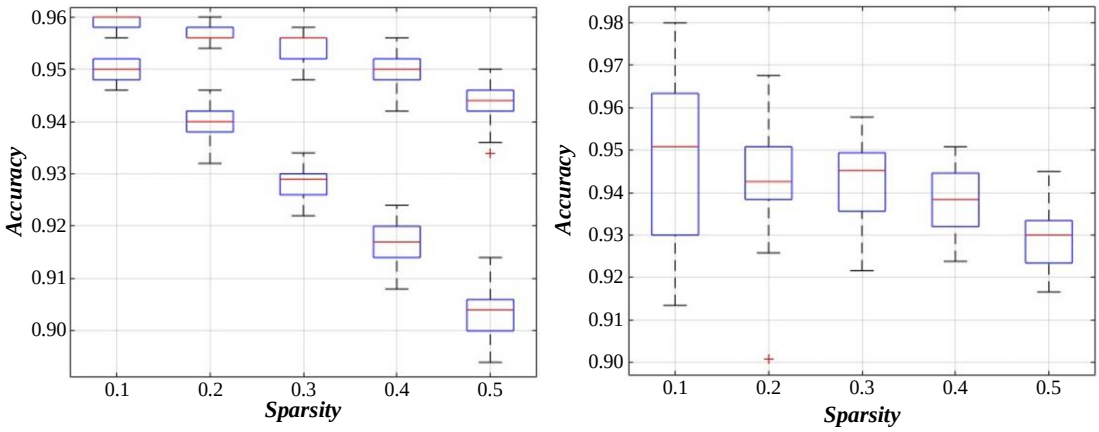

Figure 5. Plot of sensitivity versus *p*.

100 μm was determined as the best PMA treatment concentration. It can effectively inhibit the amplification of dead bacteria DNA without affecting the PCR process of live bacteria DNA. At the same time, it takes into account the control of experimental cost. It is an ideal choice to accurately distinguish the status of live and dead bacteria.

Figure 6 shows the experimental results of FPL and SWT. Among them, the *Ct* values at 0 min were similar between the blank group (N group) and the group that added 0.1 μm PMA but did not undergo time exposure, confirming that the addition of PMA itself did not have a direct effect on the detection results of qPCR. In the 9 different PMA-treated groups setup, Ct values peaked at 10 min as the exposure time increased, and then slowly decreased as the exposure time was further extended. This

phenomenon suggests that a 10-minute exposure time is the optimal time to inhibit the amplification of DNA-like DNA in dead bullae at a PMA concentration of 100 μm, as longer exposures may unnecessarily interfere with the detection of viable bacteria. Therefore, after comprehensive consideration, we determined that 10 min is the most appropriate exposure time, which can not only ensure the efficiency and specificity of PMA treatment, but also effectively avoid potential interference with subsequent viable bacteria testing.

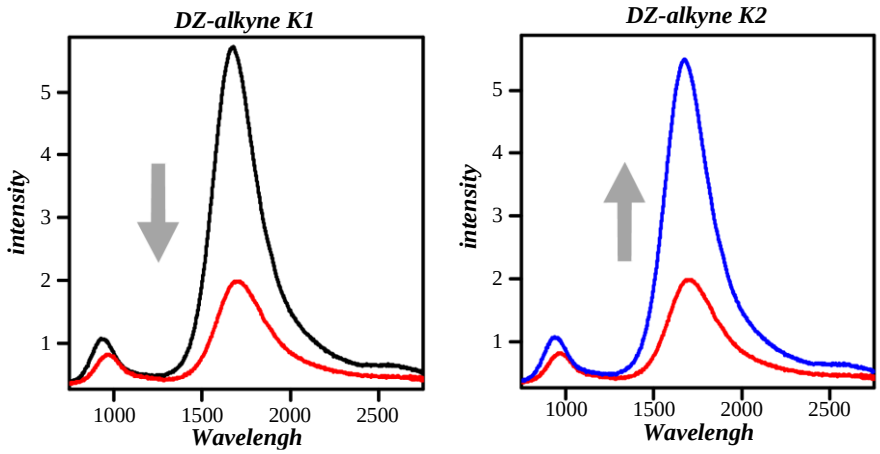

Figure 6. FPL and SWT.

In order to verify the effectiveness of the new assay for the detection of live bacteria under optimal PMA (azide DNA dye) treatment conditions, a concentration of 0.5 μg/mL of PMA was used in the experiment. In the experiment, a bullous mixed bacterial solution containing different proportions of viable bacteria gradually decreased from 100% to 0%, and one group of bacterial solutions was treated with 0.5 μg/mL PMA for qPCR detection as the PMA-qPCR group. The other group was directly tested by qPCR without PMA treatment as the control qPCR group. At the same time, the same proportion of viable bacteria and treated with 0.5 μg/mL PMA was prepared with 2216E liquid medium as an additional control group (2216E group). By comparing the differences in the test results between the groups, the aim was to comprehensively evaluate the accuracy and reliability of the new method in accurately distinguishing between the status of live and dead bacteria.

Figure 7 shows the absolute PL profiles of A and B, where the *Ct* values of conventional qPCR are not affected by the change in the proportion of viable bacteria in the template, which clearly points to the limitations of conventional qPCR methods that cannot distinguish between live and dead DNA. In contrast, when pretreated with 0.5 μg/mL PMA (PMA-qPCR group) and the control group (2216E group) with the same proportion of viable bacteria prepared with 2216E liquid medium and treated with PMA, the *Ct* values of both groups showed a significant downward trend with the increase of the proportion of viable bacteria, and the trends of the two groups were highly consistent, and the values were very similar. This result strongly demonstrates that the PCR method after PMA treatment can effectively detect live bullous bacteria and has the ability to accurately distinguish between live and dead bacterial DNA.

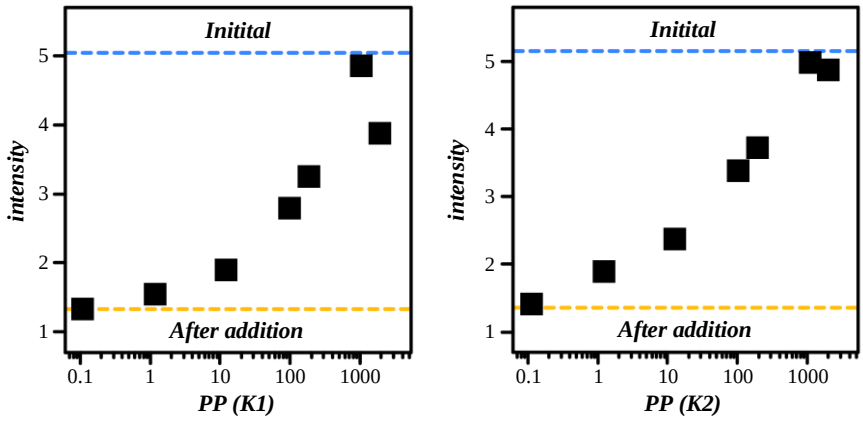

Figure 7. A, B absolute PL spectra.

Under optimized PMA treatment conditions, serial concentrations (6.625 CFU/mL to 1.325×10^7 CFU/mL) of bullous standard strains were detected by qPCR versus ddPCR with four replicates of each concentration. **Figure 8** shows the analysis of GC content and homopolymer function. Within the experimental range of the new qPCR ($R^2 = 0.9973$) and ddPCR ($R^2 = 0.9965$) methods, there is a strong correlation between the number of viable bacteria and Ct value or ddPCR copy number. Regression analysis showed that the LOD value of the PMA-ddPCR method was 29.33 CFU/mL, while the LOD value of the PMA-qPCR method was 1.14×10^3 CFU/mL. This indicates that the optimized PMA-ddPCR method is more sensitive in detecting bullous classes at low concentrations.

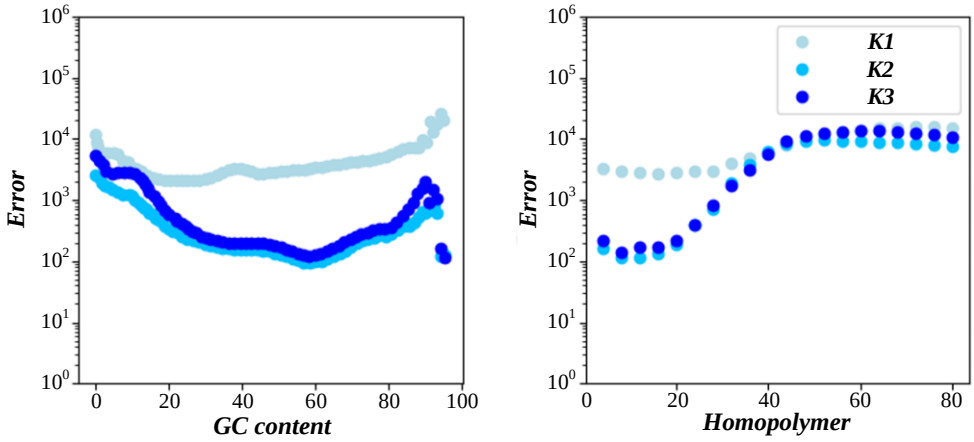

Figure 8. GC content and homopolymer function analysis.

Through the regression equation (PMA-qPCR: $Y = -3.528X + 48.93$; PMA-ddPCR: $Y = 0.9368X - 2.033$), Youden index and cut-off value analysis, the LOQ value of PMA-qPCR method in pure culture bacterial solution was calculated to be 1.29×10^3 CFU/mL, and the LOQ value of PMA-ddPCR method was 53.64 CFU/mL, as shown in **Table 2**. The optimized PMA-ddPCR method is more sensitive to the detection of bullous species at low concentrations.

Table 2. LOQ values of PMA-ddPCR method.

Plate count (CFU/mL)	PMA-qPCR (*C* value)	PMA-ddPCR (Copies/uL)
15.96	28.308	6908.82
1596	33.192	607.902204
159,600	37.272	13.272
15,960,000	41.424	1.2
1,596,000,000	45.504	0.612
1,596,000,000	-	159.6

3.3. Specificity test of qPCR detection method based on comprehensive nursing

3.3.1. Biomolecule-based digital PCR reaction mixtures

In order to further explore the detection techniques of biomolecules, especially genes, and to facilitate the application of relevant advanced mathematical methods in biological research, we designed an assay to verify the specificity of the qPCR method. The assay used a variety of control materials, including wild-type (WT) and mutant (MT1) of *Campylobacter jejuni*, related plasmids, DNA from *Salmonella*, *E. coli* and other bacteria, as well as chicken DNA and nuclease-free water, aiming to: (1) strictly verify the specificity of primers and probes to ensure that the qPCR method can accurately identify target genes, which is similar to the journal's pursuit of accurate identification and identification of biomolecules; and (2) to comprehensively evaluate the ability of the method to distinguish between different mutant subtypes of *Campylobacter jejuni*, thereby confirming its great potential for direct identification of mutant subtypes.

Both ddPCR and qPCR are based on the amplification of the target gene and quantification of the fluorescence value detected by the probe, and the primer and probe can be used universally. The ddPCR reaction system was 25 μL and contained 12.5 μL of 2 × PerfeCTa qPCR ToughMix UNG, 100 nm fluorescein, 1.2 μm primers, and 250 nm probes according to the manufacturer's recommendations. Under sterile conditions, 2.5 μL of purified genomic DNA was added to the prepared PCR mixture, mixed well and briefly centrifuged. Since the sensitivity of ddPCR is 2 orders of magnitude higher than that of qPCR, and it is more susceptible to DNA contamination of miscellaneous bacteria, the preparation of PCR mixture must be strictly aseptic to ensure the accuracy of the results.

3.3.2. Optimization of reaction conditions of qPCR detection method

In the primer concentration optimization test of qPCR reaction, by setting the primer concentration gradient (600 nM to 1800 nM), it was found that the *Cq* values of plasmids pWT-gyrA and pMT2-gyrA reached the minimum at the primer concentration of 1200 nM, and the *Cq* values of 1200 nM and 1500 nM were similar for pMT2-gyrA. In view of the combined consideration of minimum *Cq* value and cost-effectiveness, the optimal primer concentration for qPCR reaction was finally determined to be 1200 nM.

Through careful optimization, the optimal concentrations of primers and probes in qPCR method were determined to be 250 nM and 1200 nM, respectively. On this

basis, the influence of annealing temperature in the range of 56 ℃ to 66 ℃ was deeply studied. The results showed that for *Campylobacter jejuni* WT and MT2 types, the *Cq* value was small and the non-specific response was low when the annealing temperature was 60 ℃ and 62 ℃; For the MT1 type, the annealing temperatures of 58 ℃ and 60 ℃ exhibit similar characteristics. Considering amplification efficiency, non-specific reaction and *Cq* value, 60 ℃ was selected as the optimal annealing temperature.

After optimization, it was determined that the optimal primer concentration for the qPCR reaction was 250 nM, the probe concentration was 1200 nM, and the annealing temperature was 60 ℃. Results of qPCR reactions performed under these conditions are shown in **Figure 9**, Biological Constraints in CIFAR-10 assay images. Through digital droplet PCR technology, critical information can be effectively retained and optimized in reaction conditions.

Figure 10 shows a comparison of ROC curves. The qPCR method can specifically detect *Campylobacter jejuni*, including wild-type (WT), mutant (MT1, MT2) and related plasmids. The method did not yield false positive results in negative controls of *Salmonella*, *Campylobacter coli*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Pasteurella*, chicken genomic DNA and nuclease-free water. This indicates that the qPCR method is particular, not only to detect C. jejuni directly but also to type it.

Figure 11 shows the CoMix linear relationship when different *Campylobacter jejuni* plasmids (pWT-gyrA, pMT1-gyrA, pMT2-gyrA) are detected by qPCR method, specifically:

$y = -3.5698x + 40.932$, with a good linear relationship ($R^2 = 0.9956$).

$y = -3.4123x + 39.33$, with a good linear relationship ($R^2 = 0.9995$).

$y = -3.4362x + 41.35$, with a good linear relationship ($R^2 = 0.9995$).

This indicates that the qPCR method shows high linear correlation in different plasmid detection and has good quantitative ability.

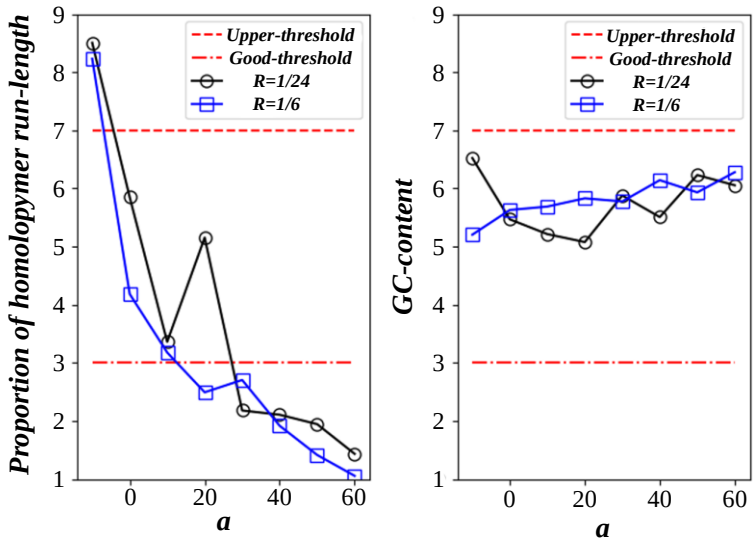

Figure 9. Biological constraints in CIFAR-10 experimental images.

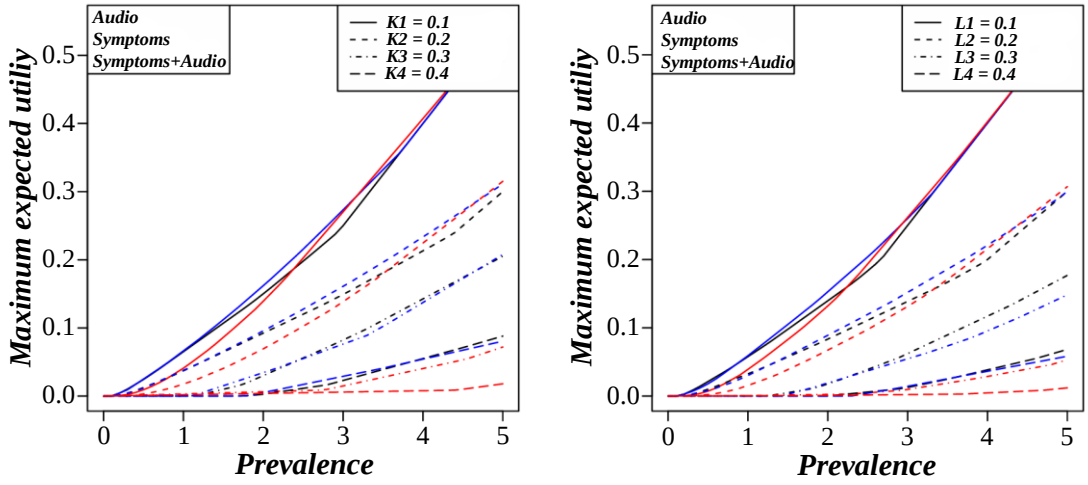

Figure 10. Comparison of ROC curves.

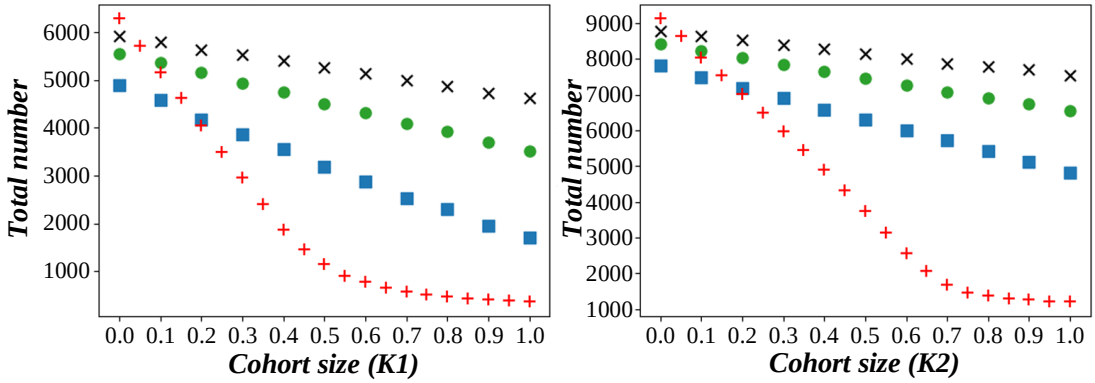

Figure 11. CoMix linear relationship.

4. Conclusion

The treatment of bullous dermatoid diseases, being a severe threat to skin health, has long been a significant challenge in dermatology. It demands an in-depth exploration of biomolecular mechanisms like Protein Structural Mechanics, Nucleic Acid Mechanics, and Protein-Protein Interactions Mechanics, as well as an optimization of treatment strategies. Although traditional treatment methods have certain advantages, they are frequently accompanied by issues such as long treatment periods and substantial side effects.

In recent years, with the rapid development of biotechnology, digital droplet PCR technology, as an advanced molecular biology detection method, has exhibited unique advantages in the treatment of bullous skin diseases. With high sensitivity, specificity, and throughput, this technology can swiftly and precisely detect genes associated with bullous skin diseases, aiding doctors in accurately diagnosing disease types and formulating personalized treatment plans to remarkably enhance treatment outcomes. Meanwhile, digital droplet PCR technology can also effectively monitor disease recurrence, offering patients more comprehensive care. In a clinical study involving 50 patients with bullous skin diseases, the area of skin lesions was on average reduced by 70%, clinical symptoms were significantly alleviated, drug side effects were mitigated, and the patients' quality of life was notably improved. This not only reflects

the clinical application value of digital droplet PCR technology but also further propels the in-depth development of biomolecular research and Cell Mechanics studies, including aspects like Cell Membrane Elasticity, Cytoskeleton Mechanics, and Intracellular Force Transmission.

ddPCR technology has great clinical significance, especially in personalized treatment. Bullous dermatoses are complex, and each patient's symptoms and response to treatment varies. With its high sensitivity and precise quantification, ddPCR technology can detect biomarkers associated with bullous skin diseases, such as BP180, Dsg1/Dsg3, etc., earlier and more accurately. Based on the results of these tests, doctors can gain timely insight into subtle changes in the patient's condition in the early stages of disease development, so that treatment can be adjusted earlier. For example, when abnormal fluctuations in biomarker levels are detected, doctors may adjust the dose of immunosuppressants or change the treatment. This early and precise adjustment of treatment plan can help patients to achieve more effective control of their condition, reduce the occurrence of complications, and significantly improve the prognosis of patients, bringing new hope and strong guarantee for the recovery of patients with bullous skin diseases.

Ethical approval: Not applicable.

Conflict of interest: The author declares no conflict of interest.

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