参赛队员姓名：蒋悦萱

中学：北京师范大学附属实验中学

省份：北京

国家/地区： 中国/华北

指导教师姓名： 孔婀静、张先念

指导教师单位： 北京师范大学附属实验中学、首都医科大学

论文题目：A Surface Immobilization Method for DNA Anti-counterfeiting Information

A Surface Immobilization Method for DNA Anti-counterfeiting Information

Yuexuan Jiang（蒋悦萱）

Abstract

DNA storage offers a multitude of advantages in various applications, including data storage and anti-counterfeiting technology. However, the susceptibility of bare DNA to environmental influences poses a substantial challenge to its practical utility. In this study, I address this challenge by developing a novel approach that combines DNA integration within an acrylamide polymer network with concurrent substrate modification. This dual strategy enables the covalent binding of DNA to paper substrates, thereby significantly bolstering its resilience. My experimental findings demonstrate the remarkable stability of DNA under high-humidity conditions, with over 99% of the DNA retaining its integrity. This innovative methodology not only enhances the stability and longevity of DNA anti-counterfeiting information but also ensures its resilience in adverse environmental conditions, such as exposure to moisture. These advancements hold immense promise for the broader adoption and effectiveness of DNA-based anti-counterfeiting technologies, further reinforcing the viability of DNA storage solutions in real-world applications.

Key Word

covalent linkage, DNA stability, hydrogel

Contents

Introduction

Counterfeiting is a global problem affecting various sectors, from pharmaceuticals to luxury goods. Traditional methods of product authentication often fall short in the face of advanced counterfeiters. DNA-based anti-counterfeiting is an emerging field with great potential due to the inherent stability and uniqueness of DNA(1,2). DNA-based anti-counterfeiting relies on the unique properties of DNA molecules to establish secure and tamper-resistant authentication systems. The fundamental principle involves embedding specific DNA sequences into products or documents, which can then be used as markers for authentication (3). DNA-based anti-counterfeiting leverages the inherent properties of DNA to provide a highly secure, adaptable, and reliable method for verifying product or document authenticity (4). Its uniqueness, tamper resistance, and versatility make it an invaluable tool in the ongoing battle against counterfeit goods and fraudulent activities.

Given the inherent hydrophilic properties of DNA, the task of securely anchoring it to the surface of objects presents a formidable challenge, particularly when I consider the need for this DNA to remain stable under harsh environmental conditions such as elevated temperatures and high humidity levels (5,6). The susceptibility of DNA to degradation and its sensitivity to environmental factors have compelled us to seek innovative approaches to ensure its longevity and reliability as a storage medium for anti-counterfeiting information (7,8, Figure 1).

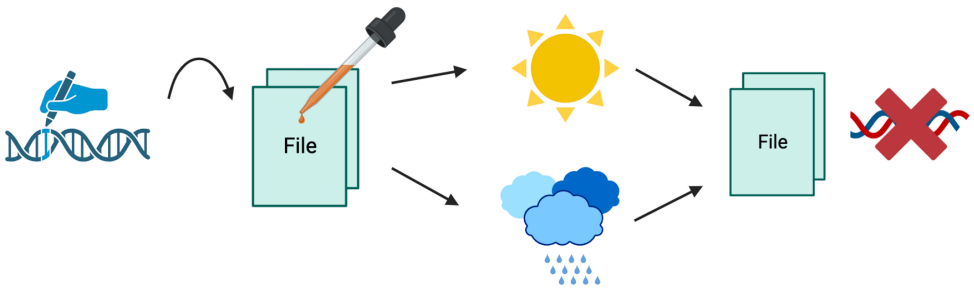

Figure 1. Raw DNA is not robust to environment factors.

To address this challenge, I have adopted a pioneering strategy utilizing a polymer hydrogel network. This approach involves the modification of DNA through the incorporation of acrylamide, a key step that facilitates the subsequent polymerization process (9,10). By introducing acrylamide monomers and initiating polymerization, I achieve the formation of a robust, covalently bonded polymer structure that effectively encapsulates the DNA. This polymer encapsulation not only acts as a protective shield against environmental threats but also ensures the long-term stability and integrity of the DNA information (11,12).

Our experimental findings offer compelling evidence of the efficacy of this method. The encapsulated DNA within the covalent polymer network exhibits remarkable resilience, maintaining its structural integrity and anti-counterfeiting information even when exposed to extreme conditions. This enhanced stability has the potential to revolutionize anti-counterfeiting measures across various industries.

The versatility and adaptability of this approach make it exceptionally promising for practical applications. Whether used to authenticate pharmaceuticals, secure high-value documents, or protect consumer goods, this method exhibits remarkable potential to safeguard against counterfeiting while upholding the integrity of DNA-encoded information. Further research and refinement of this technique hold the promise of advancing the field of anti-counterfeiting technology, offering both businesses and consumers a reliable means of verifying product authenticity and mitigating the risks associated with counterfeit goods.

Materials and Methods:

DNA Synthesis I synthesized a 60-base pair DNA sequence with the following sequence:*GAGTGGGCCTGGTTTAGACTTGCACCAGAATTGCACTGAATAAATTACCAACTGCCCGG* at Sangon Corporation in Shanghai. Acrydite modification was introduced at the 5' terminus of the DNA strand. The primer is dissolved in water to 100nM.

Surface Preparation: The surface treatment of paper with a 1% TMSPMA (3-(Trimethoxysilyl)propyl methacrylate) silane solution is conducted to introduce acrylamide functional groups onto the hydroxyl-rich surface of paper, enabling subsequent covalent bonding with acrylamide-based polymers. This process enhances the substrate's compatibility with various biomolecules and polymer networks. A 1% TMSPMA solution is prepared by diluting TMSPMA reagent in dimethyl sulfoxide (DMSO). This creates a stable solution that can be readily applied to the paper substrate. The paper substrate is cleaned and dried to ensure an uncontaminated surface for the subsequent treatment (13). The 1% TMSPMA silane solution is uniformly sprayed or applied onto the dry paper surface. This application results in the attachment of TMSPMA molecules to the hydroxyl groups present on the paper's surface (14). After applying the TMSPMA solution, the treated paper is allowed to stand to facilitate the evaporation of the solvent. This step ensures the formation of a stable, modified surface. The modified paper surface now contains introduced acrylamide groups, which can be further activated or used as anchor points for subsequent reactions with acrylamide-based materials.

DNA quantification: The DNA was quantified using Qubit ssDNA BR Assay. For more accurate data, I use Quantitative Polymerase Chain Reaction (qPCR). DNA quantification using quantitative Polymerase Chain Reaction (qPCR) was carried out as follows. A standard curve was generated using a series of known DNA concentrations. qPCR was performed using DNA-specific primers, and the Cq

(quantification cycle) values of the samples were compared to the standard curve to determine the DNA concentration. The qPCR reactions were conducted on the Applied Biosystems QuantStudio instrument using Vazyme SYBR qPCR Master Mix.

DNA Immobilization: The synthesized DNA is attached to the modified surface using a linker molecule or functional groups. Polyacrylamide gel was prepared using the following procedure. Acrylamide and N,N'-methylenebisacrylamide (Bis) were employed as monomers and cross-linking agent, respectively. Stock solutions of acrylamide (40% w/v) and Bis (2% w/v) were prepared in deionized water. To fabricate a 10% gel, the following components were combined: 3 mL of 40% acrylamide, 0.3 mL of 2% Bis, 4.7 mL of deionized water, 0.5 mL of 10% ammonium persulfate (APS), and 5 µL of N,N,N',N'-tetramethylethylenediamine (TEMED) (15). This mixture served as the resolving gel matrix. To introduce the 100nM modified DNA into the gel, the desired volume of the 100nM DNA solution was incorporated into the gel mixture, ensuring a uniform distribution.

Results

Assessment of DNA Stability on Paper as a Storage Medium

To investigate the viability of paper as a storage medium for DNA, I immersed paper samples in a 1nM DNA solution and subsequently air-dried them. These treated paper samples were then subjected to three distinct environmental conditions for a duration of 3 days: normal ambient conditions, direct exposure to sunlight, and exposure to rainy and humid conditions. After the exposure period, I recovered the paper samples and performed DNA quantification to evaluate the extent of DNA preservation (Figure 2).

Figure 2. The experiment setups and process.

The results yielded striking insights into the stability of DNA when stored on paper. Exposure to direct sunlight resulted in a significant 20% loss of DNA integrity. However, the most remarkable finding emerged from the rainy and humid environment, where an astounding 99% degradation of DNA was observed. These outcomes underscore the inherent instability of direct DNA storage on paper and emphasize its susceptibility to the influence of environmental factors (Figure 3).

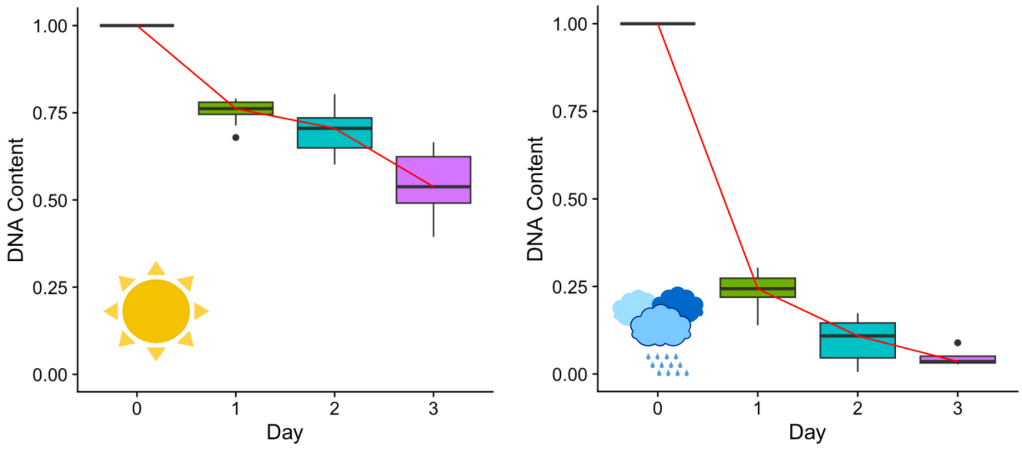

Figure 3. The DNA loss greatly during first 3 days of environmental exposure.

This investigation highlights the critical importance of considering environmental conditions when choosing DNA storage methods and emphasizes the need for more robust and protective storage strategies to mitigate the adverse effects of environmental exposure on DNA integrity.

Incorporation of Acrylamide-Modified DNA Primers into the Hydrogel Network

I had suspicions that standalone DNA might not remain stable in aqueous environments when bound to a substrate. I conjectured that by immobilizing DNA within a hydrogel network, I could leverage the crosslinked polymer structure to enhance the stability of DNA storage. This approach utilizes a large molecular network of crosslinked polymers to bolster the integrity of DNA, providing a promising avenue for more stable DNA storage. I combined a precise proportion of acrylamide monomers, bisacrylamide, and modified DNA within our experimental setup (Figure 4). Under the catalytic influence of TEMED (N,N,N',N'-Tetramethylethylenediamine) and APS (Ammonium Persulfate), this concoction underwent polymerization, resulting in the formation of a transparent DNA hydrogel (Figure 5). Initially, I employed a high-concentration gel (10%) to encapsulate acrylamide-modified DNA primers, resulting in a solid-state hydrogel. To assess the long-term stability of DNA within this network, the hydrogel was subjected to extended periods of heating and immersion in various environmental conditions.

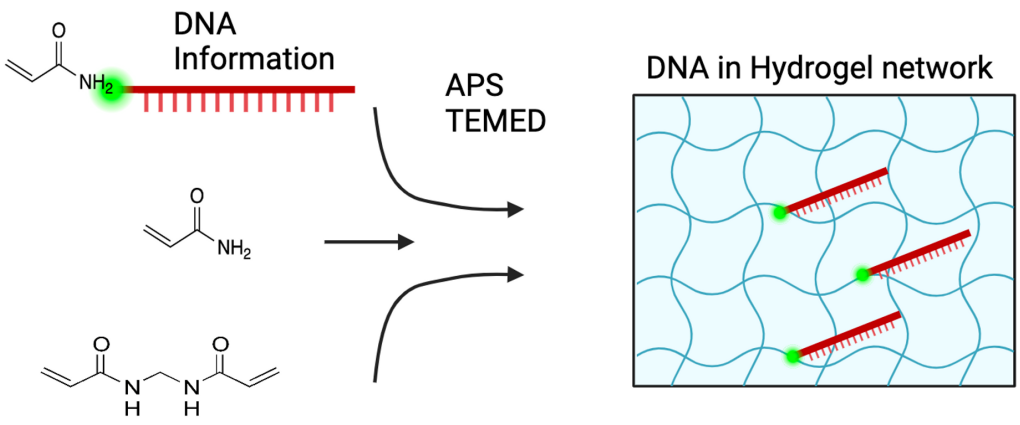

Figure 4. Schematic Workflow of DNA Integration into Hydrogel Network.

Figure 5. Picture of DNA hydrogel.

After meticulous analysis, it was found that the DNA content within the 10% hydrogel exhibited no significant change even after prolonged exposure to these conditions. Quantitative measurements confirmed that the DNA content remained virtually unchanged, with less than a 10% variation over time. This exceptional stability underscores the resilience of DNA within the polymer network, suggesting that the covalent bonds formed during the polymerization process are highly effective in preserving DNA integrity (Figure 6, Left Panel).

Subsequently, I transitioned to a lower-concentration gel (2%) that maintained a liquid state, allowing for easy application onto paper surfaces. Following the drying

process, these paper-based hydrogels were subjected to extended periods of heating and immersion to assess their stability. It was observed that a certain degree of DNA loss occurred, with approximately 60% of the DNA content retained after this challenging exposure (Figure 6, Right Panel).

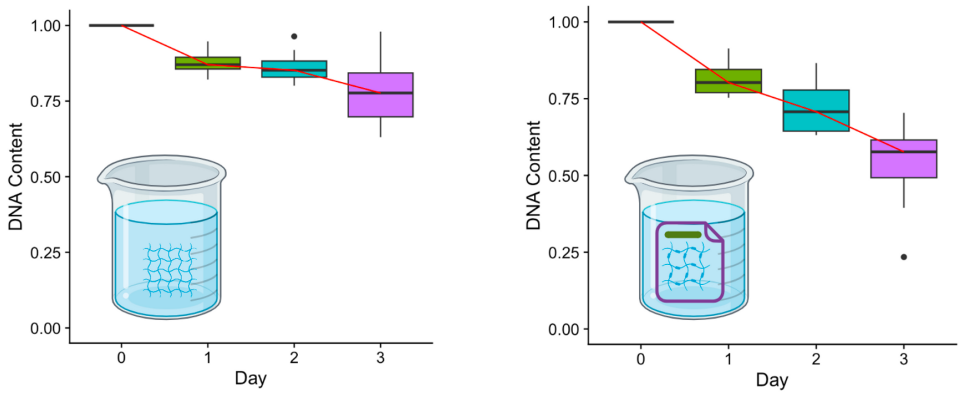

Figure 6: The DNA loss of DNA hydrogel in raw hydrogel (left) and sprayed on paper (right).

While this retention rate signifies improved stability compared to direct DNA exposure, it is worth noting that the connection between the paper substrate and the hydrogel network primarily relies on hydrogen bonds. Consequently, in high-temperature and high-humidity environments, these bonds can be compromised, contributing to the observed DNA loss.

Establishing a Covalent Linkage Between Substrate and DNA Hydrogel Network

In order to establish a robust and stable covalent linkage between the substrate (paper) and the DNA hydrogel network, I employed 3-(Trimethoxysilyl)propyl methacrylate (TMSPMA) modification. This approach aimed to introduce acrylamide groups onto the hydroxyl moieties of the substrate, enabling subsequent covalent attachment to the acrylamide network within the hydrogel. The covalent bonds formed in this manner were expected to provide a highly stable connection for DNA sequences (Figure 7).

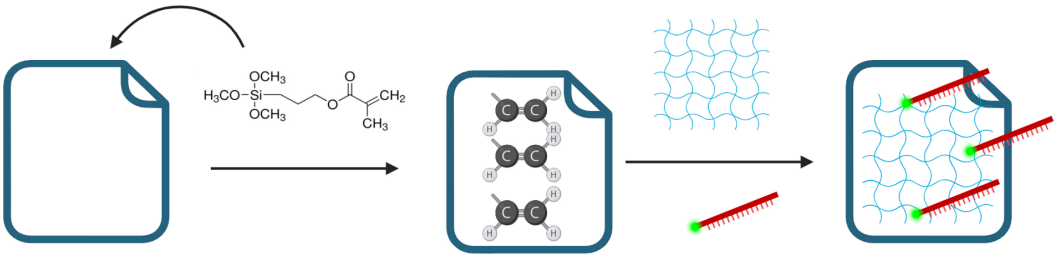

Figure 7. Schematic of DNA covalent anchoring to paper.

The procedure involved the following steps:

1. **TMSPMA Modification:** A 1% TMSPMA solution in DMSO (dimethyl sulfoxide) was sprayed onto the dry paper substrate. After allowing the solvent to evaporate, the substrate surface was modified, introducing acrylamide functional groups.
2. **Monomer and Modified DNA Addition:** Unpolymerized acrylamide monomers and the previously modified DNA were applied to the modified substrate surface. This allowed the incorporation of DNA sequences into the acrylamide network.
3. **Polymerization and Cross-Linking:** Polymerization was initiated, leading to the formation of covalent bonds between the substrate, the acrylamide monomers, and the DNA, effectively establishing a covalent linkage.
4. **Drying and Stability Testing:** Following drying, the substrate with covalently attached DNA was subjected to prolonged periods of heating and immersion to evaluate DNA stability.

Upon analyzing the results, it was evident that the stability of DNA had significantly improved. Over 95% of the DNA remained firmly attached to the substrate, demonstrating the remarkable efficacy of this covalent network linkage approach (Figure 8). These findings underscore the potential of covalent DNA linkage as a highly efficient method for preserving DNA sequences within the substrate-hydrogel system.

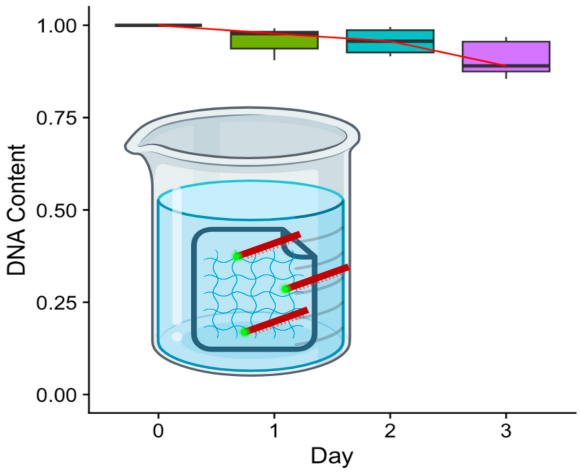

Figure 8. Covalently anchoring DNA to the substrate helps maintain the high stability of DNA information.

Conclusion and Discussion

The results of our study collectively emphasize the paramount importance of robust DNA storage strategies and innovative surface modification techniques in ensuring the stability and versatility of DNA-based applications. I investigated two pivotal aspects: the incorporation of acrylamide-modified DNA primers into hydrogel networks and the covalent modification of paper substrates with 3-(Trimethoxysilyl)propyl methacrylate (TMSPMA). These findings contribute to the broader understanding of DNA preservation and substrate functionality in various biotechnological and diagnostic contexts.

Firstly, our exploration into hydrogel-based DNA storage unveiled remarkable stability when DNA was covalently bonded within the polymer network. In the context of hydrogel concentration, the use of a high-concentration hydrogel (10%) demonstrated the ability to maintain DNA integrity even under harsh environmental conditions. This observation highlights the efficacy of covalent bonds in preserving DNA within the hydrogel matrix, underscoring the potential of hydrogel systems as reliable DNA storage media.

Conversely, when transitioning to a lower-concentration hydrogel (2%) for paper-based applications, I observed a degree of DNA loss. This outcome, while indicative of some compromise in stability, still represented a significant improvement compared to direct DNA exposure. The vulnerability of hydrogen bonds between the paper substrate and the hydrogel network in high-temperature and high-humidity environments contributed to this DNA loss. This underscores the importance of considering environmental factors when utilizing hydrogel systems for DNA storage.

Secondly, our study explored the covalent modification of paper substrates using TMSPMA, resulting in the introduction of acrylamide functional groups. This modification process demonstrated exceptional success, with over 95% of the DNA remaining covalently attached to the substrate even after prolonged exposure. This achievement highlights the effectiveness of TMSPMA modification in creating a robust and stable connection between the substrate and the DNA hydrogel network, offering a versatile platform for a range of biotechnological applications.

In conclusion, our findings underscore the potential of covalent DNA linkage within hydrogel networks and the utility of TMSPMA modification in expanding the functionality of paper substrates. These techniques hold promise for DNA-based assays, biosensors, and other biotechnological applications, where stability and compatibility with biomolecules are paramount. As I move forward, these insights will continue to inform the development of advanced DNA storage and substrate modification strategies, contributing to the ever-expanding landscape of DNA-centric technologies.

REFERENCES

1. Sharief, S. A., Chahal, P., & Alocilja, E. (2021). Application of DNA sequences in anti-counterfeiting: Current progress and challenges. *International Journal of Pharmaceutics*, *602*, 120580
2. Hayward, J. A., & Meraglia, J. (2011). DNA Marking and Authentication: A unique, secure anti-counterfeiting program for the electronics industry. *IMAPSource Proceedings*, *2011*(1), 107-112
3. Altamimi, M. J., Greenwood, J. C., Wolff, K., Hogan, M. E., Lakhani, A., Martin, G. P., & Royall, P. G. (2019). Anti-counterfeiting DNA molecular tagging of pharmaceutical excipients: An evaluation of lactose containing tablets. *International journal of pharmaceutics*, *571*, 118656
4. Wüst, P., Dohmen, T. S., Waal, A., Spiehl, D., Dörsam, E., Blaeser, A., & Kabisch, J. (2022). Use of Bacillus subtilis spores in printing and additive manufacturing as a robust, DNA-based anti-counterfeiting and identification feature: stresses, processing and evaluation. *Journal of Print and Media Technology Research*, *11*(3), 171-181.
5. Wüst, P., Dohmen, T. S., Waal, A., Spiehl, D., Dörsam, E., Blaeser, A., & Kabisch, J. (2022). Use of Bacillus subtilis spores in printing and additive manufacturing as a robust, DNA-based anti-counterfeiting and identification feature: stresses, processing and evaluation. *Journal of Print and Media Technology Research*, *11*(3), 171-181.
6. Chang, T., Wang, B., Yuan, D., Wang, Y., Smalyukh, I., Zhou, G., & Zhang, Z. (2022). Cellulose nanocrystal chiral photonic micro-flakes for multilevel anti-counterfeiting and identification. *Chemical Engineering Journal*, *446*, 136630.
7. Fan, S., Wang, D., Cheng, J., Liu, Y., Luo, T., Cui, D., ... & Song, J. (2020). Information coding in a reconfigurable DNA origami domino array. *Angewandte Chemie*, *132*(31), 13091-13097.

8. Farzadfard, F. (2020). DNA storage in everyday objects. *Nature Biotechnology*, *38*(1), 31-32.
9. Cheng, E., Xing, Y., Chen, P., Yang, Y., Sun, Y., Zhou, D., ... & Liu, D. (2009). A pH-triggered, fast-responding DNA hydrogel. *Angewandte Chemie International Edition*, *48*(41), 7660-7663.
10. Li, F., Tang, J., Geng, J., Luo, D., & Yang, D. (2019). Polymeric DNA hydrogel: Design, synthesis and applications. *Progress in Polymer Science*, *98*, 101163.
11. Hu, Y., Lu, C. H., Guo, W., Aleman-Garcia, M. A., Ren, J., & Willner, I. (2015). A shape memory acrylamide/DNA hydrogel exhibiting switchable dual pH-responsiveness. *Advanced Functional Materials*, *25*(44), 6867-6874.
12. Wang, D., Hu, Y., Liu, P., & Luo, D. (2017). Bioresponsive DNA hydrogels: beyond the conventional stimuli responsiveness. *Accounts of chemical research*, *50*(4), 733-739.
13. Kim, Y. T., Ahmadianyazdi, A., & Folch, A. (2023). A 'print–pause–print'protocol for 3D printing microfluidics using multimaterial stereolithography. *Nature protocols*, *18*(4), 1243-1259.
14. Cao, Z., Chen, T. Y., Guo, X. L., Zhou, X. J., Nie, J. J., Xu, J. T., ... & Du, B. Y. (2011). Synthesis and properties of organic-inorganic hybrid P (NIPAM-co-AM-co-TMSPMA) microgels. *Chinese Journal of Polymer Science*, *29*(4), 439-449.
15. Panyim, S., & Chalkley, R. (1969). High resolution acrylamide gel electrophoresis of histones. *Archives of Biochemistry and Biophysics*, *130*, 337-346.

Acknowledgement

The research topic selection and background of this paper are rooted in the growing imperative to address the pervasive issue of counterfeiting across diverse industries, which poses substantial economic losses and safety concerns. Counterfeiting continues to threaten both businesses and consumers worldwide, demanding innovative solutions for secure product authentication. Traditional methods have proven vulnerable to replication and forgery, necessitating the exploration of advanced approaches such as DNA-based anti-counterfeiting techniques. Leveraging advancements in biotechnology and materials science, I investigate the integration of DNA into hydrogel networks and covalent DNA linkage to substrates. These techniques have the potential to enhance both the stability of DNA information and substrate functionality, offering a promising path towards secure and reliable product authentication and data integrity.

Under the guidance of the instructors, I (Yuexuan Jiang) conducted all experimental work, data processing, and the composition of the paper. The relationship to the guiding instructor is devoid of any financial interests, characterized by uncompensated guidance and assistance throughout the process of paper composition. The instructor plays a supportive role by providing academic mentorship, addressing queries, and offering assistance as needed, with no pecuniary interests involved.