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Article

PVA/MB-ssDNA/MXene hydrogel synthesized by freeze thawing process with the effect of MB-ssDNA

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Abstract: Freeze-thawing plays a vital role in enhancing materials in medicines. Here, we describe the F-T process of synthesis of Poly (vinyl alcol)- Methylene blue single strand-Mxene (PVA–MB-ssDNA –Mxene), which may be effective for gen delivery applications. The PVA –MB-ssDNA –Mxene hydrogel was formed using 1,3,5 consecutive cycles. We also demonstrated that PVA –MB-ssDNA –Mxene hydrogel can be formed by the affection of DNA with PVA and the MXene network. The F-T process shows the new intra molecular bond of PVA-PVA, compared to the non F-T hydrogel which formed by a biologic crosslinking as MB-ssDNA. Scanning electron microscopy reported that the microstructure. The differential scan shows three endothermic peaks at 70, 180, and 300 °C for water loss and decomposition. The swelling behavior rapidly increased due to the PVA chains in the F-T methods and then became stable. With a high concentration of MB-DNA, the tensile strength was slightly high, and the swelling behavior was low. Our results indicated that the PVA –MB-ssDNA –Mxene hydrogel using F-T process would have more suitable structural features as gene hydrogel carrier which need greater mechanical strength and stability in body analyses.

Keywords: hydrogel; MXene; MB-ssDNA; freeze thawing; physical crosslinking

1. Introduction

Freeze thawing is a critical process used extensively in the fields of medicine and healthcare. This technique involves freezing a substance, followed by thawing at a later stage [1]. This cycle is repeated multiple times, allowing for the preservation and utilization of various medical components such as proteins, cells, tissues, and organs [2]. The freeze–thaw technique has been employed to develop hydrogels for medical materials. The advantages of this freeze-thaw technique compared to the conventional technique are that it does not require a high temperature or any extra chemicals as crosslinking agents that may cause toxicity [3,4]. On the hand, one of the important challenges in using gene delivery is protecting DNA during the delivery process, optimal control of DNA release from carriers, and proper cellular uptake of DNA. This technique allows for the enhanced delivery, protection, controlled release, and cellular uptake of DNA [5,6]. DNA hydrogels are three-dimensional networks formed by self-assembling DNA strands. These materials exhibit exceptional biocompatibility, biodegradability, and programmability, making them an ideal choice for various biomedical applications. However, to fully harness their potential, it is essential to optimize their structure and properties. This is where the freeze-thaw method comes into play. In this article, we will explore the significance of freeze-thawing PVA –MB-ssDNA –Mxene in the medical field. Zhao et al. prepared a flame-retardant

PVA/PA/MXene hydrogel coating via a freeze–thaw cycle [7]. This coating showed excellent self-healing properties and high water retention (water content ≥ 90%) owing to the increased number of hydrogen bonds with the introduction of MXene. Many studies have been conducted on PVA-based hydrogels using the freeze-thaw process in the medical industry. Waresindo et al. showed that a polyvinyl alcohol (PVA) hydrogel loaded with guava leaf extract (GLE) could be fabricated by freeze–thaw (F-T) method as a wound dressing with good antibacterial activity [8]. In 2008, the effect of DNA on the mechanical properties of nanofiber hydrogels was demonstrated. PVA nanofiber gels incorporating double-stranded deoxyribonucleic acid DNA were fabricated without the aid of cross-linkers using electrospinning. Unlike the weak connection between DNA and PVA, the elastic modulus of the DNA/PVA gels was higher than that of the PVA gel [9]. In this study, we synthesized a PVA –MB-ssDNA –Mxene hydrogel using the freeze-thawing method, which can be used as an effective hydrogel approaches carrier in gene delivery research using single-stranded DNA (ss-DNA). So, we investigated PVA –MB-ssDNA –Mxene synthesis using the F-T method to study the structural, morphological, and thermal properties of this hydrogel compared to the synthesis hydrogel of PVA –MB-ssDNA –Mxene without F-T method. Due to the fact that biological cross-linking as MB-ssDNA is used in both methods, we have tried to investigate the effect of freeze-thaw cycles on the structure and increasing the tensile strength of the hydrogel. The results show that the samples were successfully prepared during different F-T processes (1, 3, and 5 cycles). The PVA –MB-ssDNA –Mxene hydrogel synthesized by the F-T method was also stronger than PVA –MB-ssDNA –Mxene without F-T Methods The. Our study also showed the effect of MB-ssDNA on the increasing the tensile strength and reducing the swelling properties of PVA –MB-ssDNA –Mxene hydrogel structure synthesized by freeze-thawing method. As a result, we introduced the novel synthesis of PVA –MB-ssDNA –Mxene hydrogel structure by the F-T method, which was influenced of biological (MB-ssDNA) and physical cross-linking which can be used as a future reliable gene carrier based hydrogel in medical purposes.

2. Experimental

2.1. Chemicals and reagents

Lithium fluoride (LiF), Ti_3AlC_2 (powder, 200 mesh), Tris Buffer, Poly (vinyl alcohol), and borax (sodium tetraborate decahydrate, purity > 99.5%, $Na_2B_4O_7$, $10H_2O$, Mw = 381.37 g/mol) ammonia water were purchased from Sigma-Aldrich. All the chemicals were used directly without further purification. Single-strand DNA such as MB-ssDNA; 5'- Atto MB_2-TCA ACA TCA GTC TGA TAA GCT A –(OH) 3’ was synthesized from Metabion (German).

2.2. Methods

2.2.1. Preparation of Ti_3C_2Tx MXene Nanosheets

$Ti_3C_2T_x$ MXene nanosheets were prepared by modified hydrofluoric acid etching. In brief, 2 g Ti_3AlC_2 powder and 2 g LiF were dissolved in 20 mL HCl solution (9 M), injected with nitrogen for deoxygenation, and sealed in an oven at 200 ℃ for 24 h.

The resulting suspension was collected and washed again. Ti_3C_2 was collected by centrifugal washing. Finally, the supernatant was freeze-dried to obtain MXene (Ti_3C_2) nanosheets.

2.2.2. Preparation of PVA –MB-ssDNA –Mxene nanocomposite hydrogel

The synthesized was modified by PVA–Mxene- borax protocol [10]. After completely dissolved 0.6 g PVA (10%w/v) at 90 ℃ for about 4 h, 6 mg/mL of ss-MB-DNA were dissolved in 16 mL distilled water and stirred continuously at 30 ℃ until DNA was completely dissolved without denaturation. Subsequently, a certain amount of MXene was added to the solution and stirred for 5 h. The mixture was cast onto a petri dish, followed by freezing at −20 ℃ for 18 h, and thawed at room temperature for 6 h. The experiment was conducted for 1, 3 and 5 consecutive cycles, respectively (**Figure 1**). The hydrogel was stored in a desiccator to prevent moisture adsorption. In parallel, we synthesized non F-T PVA –MB-ssDNA –Mxene hydrogel as described above but without the consecutive cycles.

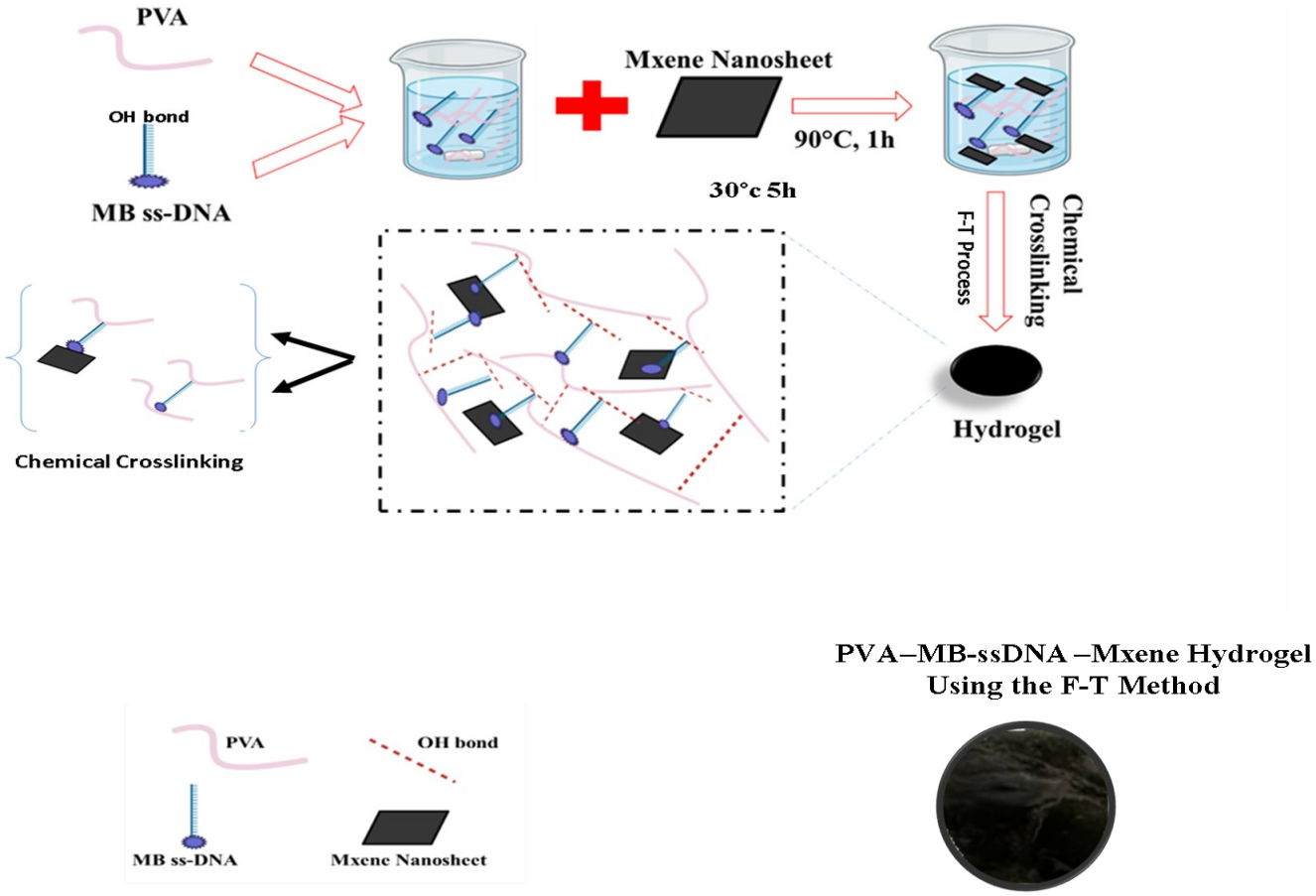

Figure 1. Step-wise preparation of PVA –MB-ssDNA –Mxene hydrogel using the freeze thaw process.

2.2.3. Swelling behavior

To investigate the swelling behavior of the synthesized of PVA –MB-ssDNA –Mxene with freeze thawing method, the samples were cut into 1 cm^2 × 1 cm^2 pieces with the same weight (W_d) and immersed in deionized water (DI) for 24 h. Samples were removed from the solution, dried with filter paper to remove excess water, and

weighed (W_w). The samples were then investigated using the following equation, and the data were reported as the statistical average and standard deviation.

$$Swelling\ (\%) = \frac{Ww - Wd}{\text{wd}} \times 100$$

where W_w is the weight of the swollen sample and W_d is the initial weight of the hydrogel.

2.2.4. Fourier transform infrared (F-TIR) characterization of the samples

A Fourier-transform infrared spectrometer (SPECTRUM ONE, Perkin Elmer, USA) was used to determine the chemical structure. The sample was scanned from 400 cm^{-1} to 4000 cm^{-1} at room temperature in air mode at a resolution of 4 cm^{-1}.

2.2.5. Scanning electron microscope (SEM)

The morphology was determined by scanning electron microscopy. In detail, the micromorphology of PVA –MB-ssDNA-MXene hydrogel by freeze thawing processes and non-freeze thawing process composite hydrogel was analyzed by FEI Inspect F50 scanning electron microscope (SEM, Quanta 250 microscope, Japan). Hydrogels were treated with liquid nitrogen to expose the inner structure. Then, hydrogel samples were rapidly dropped onto a gold-coated high purity copper block cooled in liquid nitrogen due to entering the rapid freezing to minimize compression of the hydrogel samples. In the following, we used as the airlock transfer adapter to remove the hydrogel sample from liquid nitrogen immersion and transferred to the sample preparation airlock precooled to −175 ℃.

2.2.6. Differential scanning calorimetry (DSC)

Thermal behavior of PVA –MB-ssDNA –Mxene hydrogel was determined using DSC (NETZSCH DSC 204 F1 Phoenix, Germany). The samples were placed in aluminum pans at a flow rate of 40 $mL \cdot min^{-1}$. The temperature was fixed at 30 ℃ to 350 ℃ at a flow rate of 10 $℃ \cdot min^{-1}$. The data are presented as the glass transition temperature, melting temperature, and specific heat capacity. By passing a nitrogen gas stream from 0–600 ℃ at a heating rate of 20 ℃/min, samples were scanned and thermograms were collected.

2.2.7. Tensile testing

The samples (1 mm thickness) for the tensile tests were cut with a dumbbell cutter (Analyzer Texture XT2i, Iran). The speed of the test was 10 mm/min to obtain the tensile stress (r)–strain (DL/L_0) curve, where r was calculated using the cross-sectional area of the unreformed gel. DL and L_0 indicate the deformation of the gel and initial length before deformation, respectively.

3. Results and discussion

PVA –MB-ssDNA –Mxene hydrogel was successfully prepared using the freeze-thaw technique. It exhibits a reformable shape. **Figure 2** illustrates the F-TIR spectra of the PVA –MB-ssDNA –Mxene hydrogel prepared by the freeze-thaw technique. No significant changes in the functional groups of the hydrogels were observed based on the variation of consecutive cycles in the freeze-thaw process. The functional groups of the hydrogels were similar for all compositions of hydrogel PVA –MB-ssDNA –Mxene without the F-T process based on the F-TIR analysis that was used to

qualitatively analyze the presence of functional groups in the hydrogels. In **Figure 2**, the characteristic peak at 3445 $cm^{-1}$was observed. This indicates the presence of an OH-stretching group (hydroxyl group). It refers to the presence of polyvinyl alcohol, MXene, and MB-DNA. This was in agreement with our work for synthesizing MXene-PVA hydrogel without F-T processes. It also clearly showed the major peaks related to freeze-thawed PVA (10). Another peak was attributed to the C–H stretching vibrations at 1410 cm^{-1} and 2911 cm^{-1}. F-TIR peaks at 2225 cm^{-1} and 1600 cm^{-1} were observed sequentially for the C–N stretching and C=O stretching groups. This can be created by the adsorption of MB on MXene and PVA. This is in agreement with a previous study by Zhang on MB adsorption on MXene based on electrostatic forces [11]. Another peak at 1579 cm^{-1} and the C–H bond can imply the bond between MB and PVA or MXene. In this study, the F-TIR peaks at 500 cm^{-1} and 1350 $cm^{-1}$and 1575 cm^{-1} were related to C–O, C–N, and N–H bonds of DNA. Generally, our results showed that there is no significant change on functional group of hydrogels during on variation of consecutive cycle in F-T process. As F-TIR analysis indicated the qualitatively analyze the existence of functional group of hydrogels, it can be concluded that functional group of hydrogels was still similar for all compositions of hydrogel. On the hand, F-T hydrogel results were also similar to the Non F-T MXene-PVA/MB-ssDNA hydrogel, with the difference in the shifting of the C-C and CH_2 at 800 $cm^{-1}$and 900 cm^{-1}. On the hand, results indicated F-TIR peaks at 1220 cm^{-1}at the C-O-C bond between the intramolecular PVA chains with the highlighting of the new peaks at 1141 cm^{-1} and 2882 cm^{-1}, which indicate the intra-or intermolecular bonds of PVA. These results showed that the existence of a physical process besides cross-linking (MB-ssDNA) can lead to the formation of more intra- and intermolecular PVA networks., in comparison with the conventional hydrogel methods of MXene-PVA/MB-ssDNA.

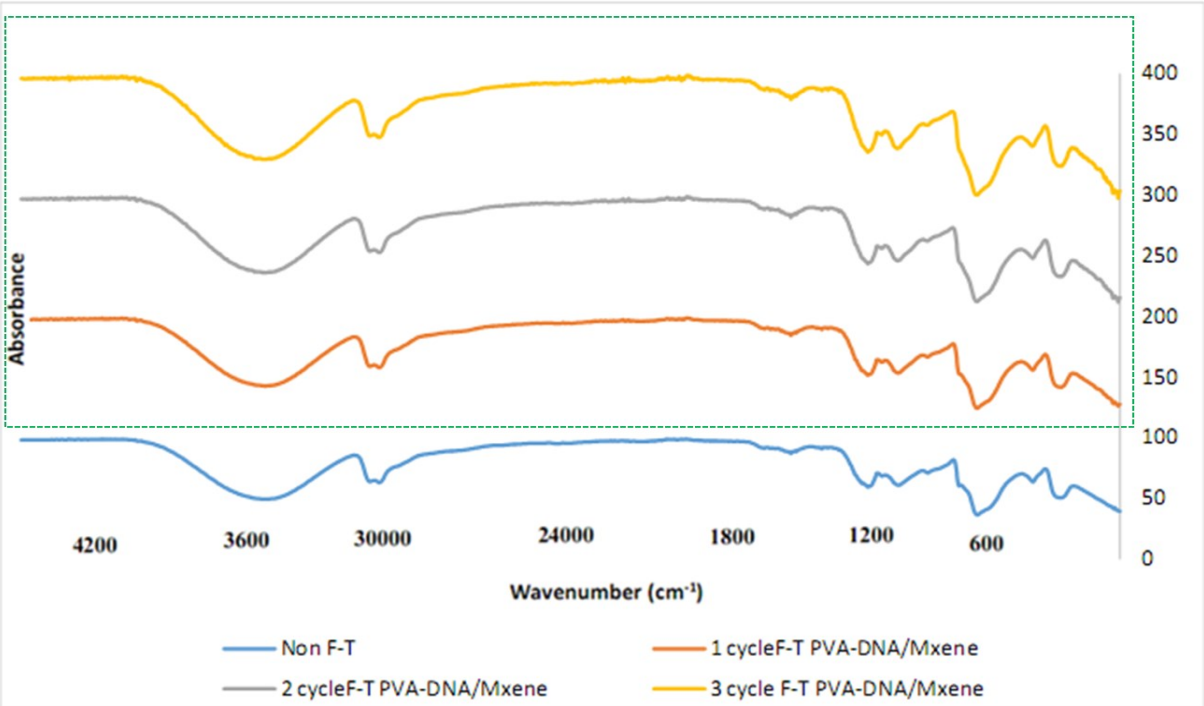

Figure 2. F-TIR spectra of PVA –MB-ssDNA –Mxene hydrogel prepared with/out F-T process.

Figure 3 shows the morphological properties of the PVA –MB-ssDNA –Mxene hydrogel could prepared using also the freeze-thaw technique. Our results revealed a porous structure. It was remarkable to note that all microstructural images presented the porous structure about forming of hydrogel by F-T methods similar non F-T method.

The pores are interconnected and regularly distributed. The pores were mostly caused by the F-T process which can show the existence of more obvious pores than non F-T method. Moreover, it seems that with three and five consecutive cycles, the number of pores was less than that with one consecutive cycle. The surface became homogenous when compared to that of the control. This implies that the hydrogel was well packed. With a high number of consecutive cycles of freeze thawing, the crosslinking reaction between DNA and MXene or PVA was successfully prepared. Our results may provide a reliable method for synthesizing DNA hydrogel carriers. Also, our results show that the presence of porous and dense structures of PVA –MB-ssDNA –Mxene using without freeze-thaw technique is more than the consecutive cycles of freeze-thaw technique. This is in agreement with a study by Sornkamnerd et al. on synthesize the tough and porous hydrogels by simple lyophilization of LC gels [12].

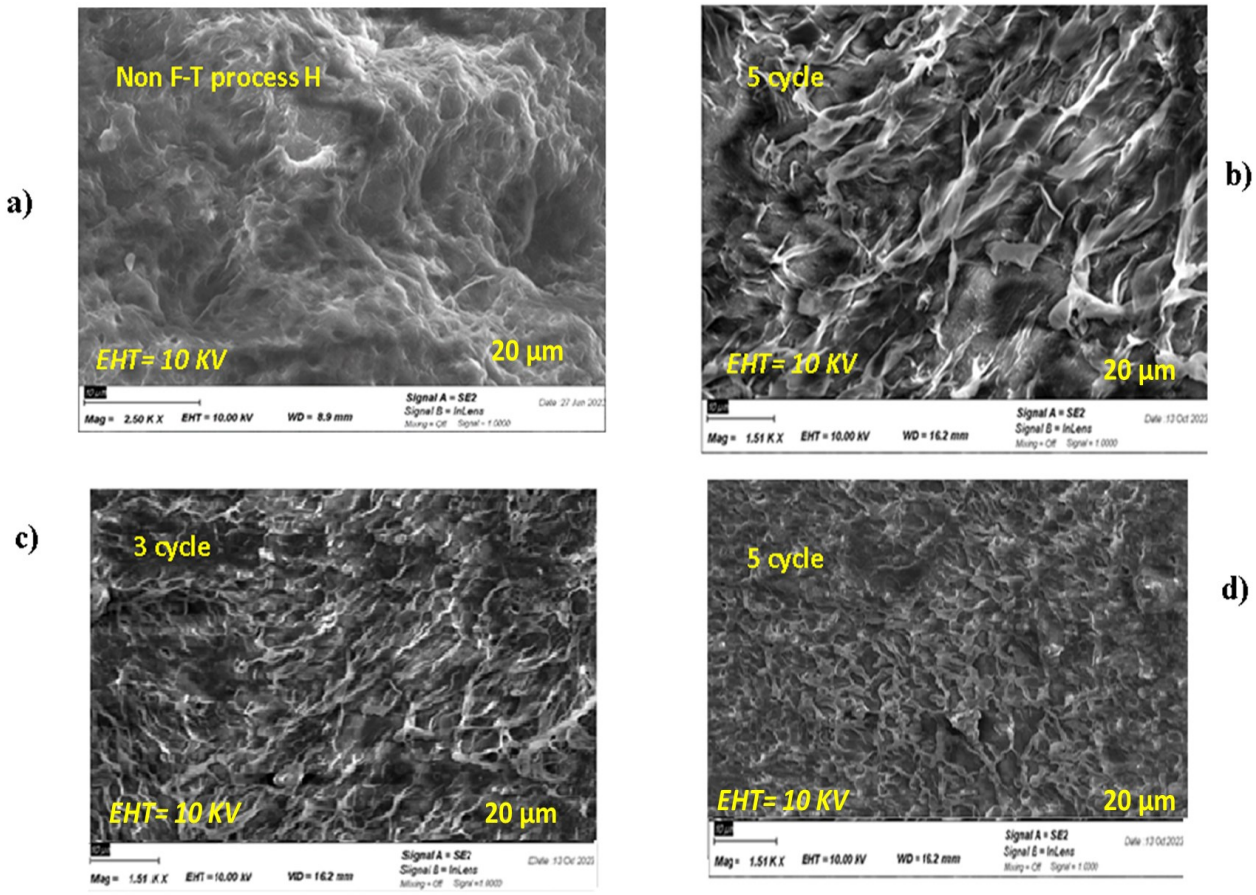

Figure 3. Morphological properties of PVA –MB-ssDNA –Mxene hydrogels. **(a)** PVA –MB-ssDNA –Mxene hydrogels synthesized without F-T methods; **(b–c)** PVA –MB-ssDNA –Mxene hydrogels synthesized with F-T processes.

Differential scanning calorimetry was performed to detect the presence of water molecules in the hydrogel network owing to the large amount of water in the hydrogel. These results showed the water state change in the MXene - and PVA-based hydrogel networks in previous studies. **Figure 4** showed the DSC measurements of DNA, polyvinyl alcohol, and the MXene hydrogel prepared by the freeze–thaw technique. Various compositions of DNA, PVA, and MXene were evaluated based on 1, 3, and 5 consecutive freeze-thaw cycles. All curves were reported in a similar form. The DSC thermogram of of MXene-PVA/MB-ssDNA hydrogel showed a small peak at 70 ℃ and then two large endothermic at approximately 180 ℃ and 300 ℃. DSC characterization confirmed the formation of the new hydrogel. Crosslinked matrices of PVA –MB-ssDNA –Mxene revealed higher thermal stability than MB-DNA. The DSC peaks at 70 ℃ indicate the water loss from the matrices, which was followed by decomposition at approximately 180 ℃ and 300 ℃. Clearly, our hydrogel fabrication method yields thermally stable cross-linked matrices of MB-DNA. DSC results also slightly shifted to 330 ℃ for three and five consecutive cycles. The presence of more PVA-PVA network may enhance the compactness of the hydrogel by the freeze–thaw process. These findings are in good agreement with the F-TIR results.

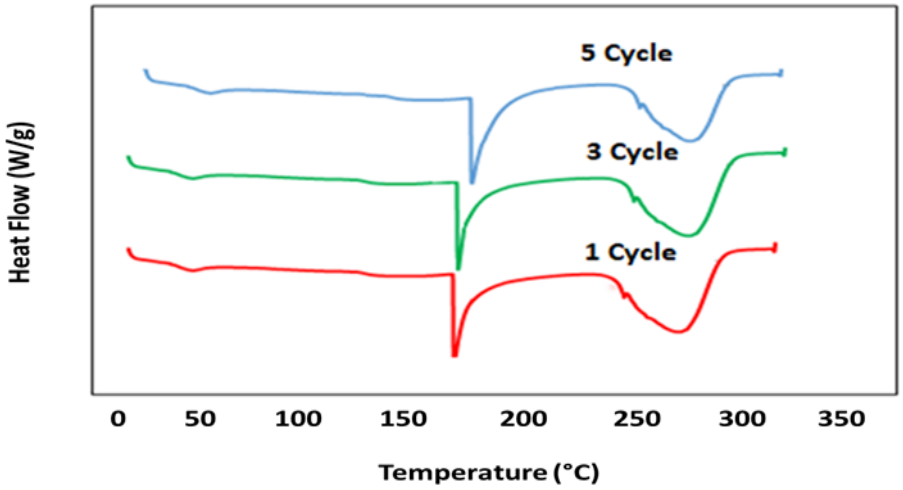

Figure 4. Thermal properties of PVA –MB-ssDNA –Mxene hydrogel composite prepared by freeze thaw process.

Figure 5 showed the swelling characteristics of the PVA –MB-ssDNA –Mxene hydrogel composite. The swelling ratio of the hydrogel structure was observed within 200 min. Subsequently, the swelling ratio remained constant. The swelling behavior can be explained by the hydrophilicity of PVA located inside the hydrogel network. They can adsorb water molecules from the system. These results are consistent with those of a study by Asy-Syifa [13]. As MB-ssDNA contains methylene blue, it can affect the swelling behavior of the PVA –MB-ssDNA –Mxene hydrogel. Martinez et al. showed the swelling and adsorption of MB in a poly (*N*, *N*-dimethylacrylamide-*co*-2-hydroxyethyl methacrylate) hydrogel [14]. MB-ssDNA, as a crosslinker, can reduce the swelling ratio. Our results showed that, as the concentration of MB-ssDNA increased, the swelling rate of the PVA –MB-ssDNA –Mxene hydrogel decreased (**Figure 6**).

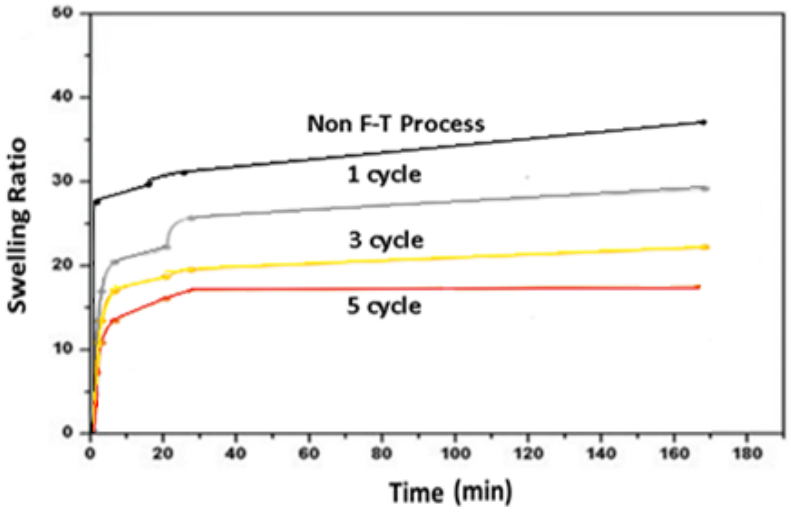

Figure 5. Swelling behavior of PVA –MB-ssDNA –Mxene hydrogel composite prepared by freeze thaw process compare to Non F-T process.

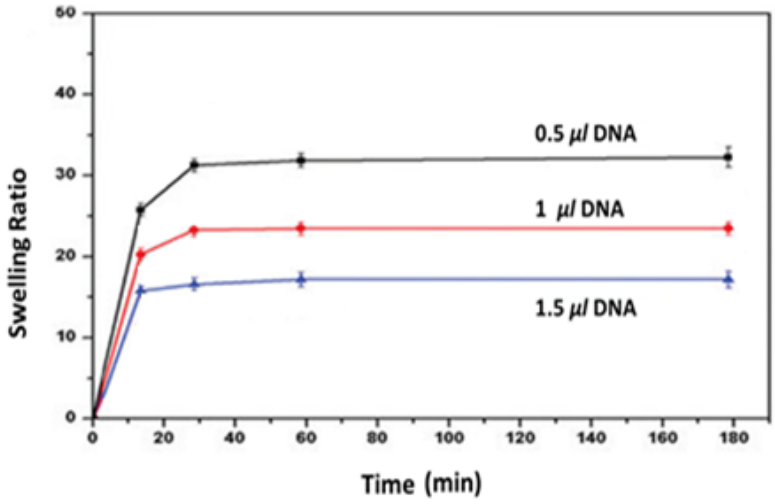

Figure 6. Swelling behavior of PVA –MB-ssDNA –Mxene hydrogel prepared by freeze thaw process with the effecting of MB-ssDNA.

Generally, the swelling behavior is associated with the interconnected porous structure in the hydrogel network, as suggested by Yu et al. [15]. Our results agree with those of the SEM analysis. In addition, the swelling (%) increased as the number of cycles increased to five. At a higher number of cycles (five), the swelling (%) rapidly increased within 30 min and reached equilibrium in approximately 50 min. The blockage of active sites on PVA chains results in a decrease in hydrophilic groups [16]. On the one hand, the swelling ratio of the hydrogel synthesized by non F-T process was lower than the nonporous hydrogels prepared by freeze–thawing of the original hydrogels. This is also in agreement with a study by Sornkamnerd [12].

Analysis of the mechanical properties of hydrogels for medical use is very important. **Figure 7** showed the tensile strength of the PVA –MB-ssDNA –Mxene hydrogel composite. Our measurements were tested, and the data were reported. A uniform structure of PVA –MB-ssDNA –Mxene was prepared by F-T processes, as shown in **Figure 2**. We tested the four measurements and the datas were also evaluated by statistical average and standard deviation.

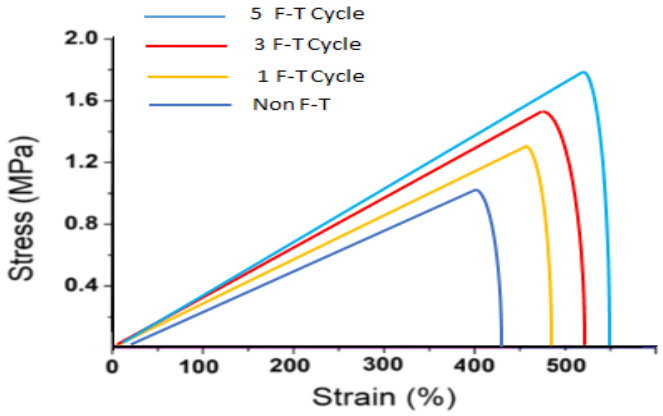

Figure 7. Tensile strength of MB-ssDNA hydrogel prepared by freeze thaw process with the effecting of MB-ssDNA.

Here, the level of tensile strength for all hydrogels by the F-T process in three cycles was in the region of 2.1 MPa to 2.5 MPa. This indicates that the PVA –MB-ssDNA –Mxene hydrogel can be prepared using the F-T technique. Based on our parallel study, it has been shown that MB-DNA can act as a biological cross-linkage which it could increase the tensile strength. As a result of the current study, the F-T process was also influenced by MB-DNA. This indicated that the hydrogel structure with the F-T method was stronger than that without the F-T method. On the hand, the tensile stress can be affected by an increase in the MB-ssDNA concentration, indicating that MB-ssDNA plays a significant role in the enhancement of mechanical strength. In fact, the PVA –MB-ssDNA –Mxene gel was robust, for example, 2 µL MXene-PVA / MB-ssDNA (**Figure 8**).

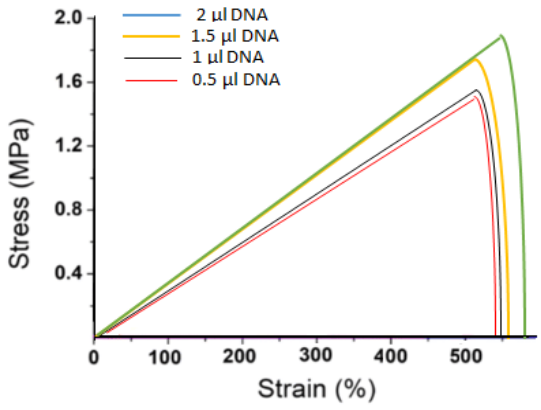

Figure 8. Tensile strength of MB-ssDNA hydrogel prepared by freeze thaw process.

4. Conclusions

The freeze-thawing method is a versatile and straightforward technique for synthesizing hydrogels. This method offers flexibility in terms of tailoring the properties of the hydrogel according to specific requirements, making it highly suitable for various biomedical applications [17,18]. DNA hydrogels are three-dimensional structures composed of crosslinked DNA molecules. These materials possess unique properties such as high water content, biocompatibility, and the ability

to respond to external stimuli like temperature variations. However, obtaining hydrogels with precise characteristics and performance remains a challenge. This is where the freeze-thawing method comes into play [19]. In this report, we successfully synthesized the PVA –MB-ssDNA –Mxene hydrogel using the freeze-thaw technique in addition to the effect of MB-ssDNA. Five consecutive freeze-thaw cycles were optimal for hydrogel formation compared to the conventional methods. Fourier transform infrared spectroscopy confirmed that hydrogen bonding resulted in a new inter-and intramolecular network of PVA-PVA throughout the hydrogel network by the OH group affected by MB-ssDNA. Scanning electron microscopy revealed the microstructure of the hydrogel. It presented as a porous network with an increase in DNA concentration, the swelling rate decreased, whereas relatively increase the tensile stress behavior occurred. Also, we showed that freeze-thaw processes can form stronger hydrogels of PVA –MB-ssDNA –Mxene with less swelling behavior compare the non F-T processes. Generally, the PVA –MB-ssDNA –Mxene hydrogel prepared with F-T method exhibited extraordinary properties for use as a medical material.

Authors contributions: Provide draft and methodology, EG; provide some of methodology and editing, SA. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest: The authors declare no conflict interest.

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