



Research article

Composite fibrin sealant reinforced with halloysite nanotubes

Martina Maria Calvino^a, Giulia D'Agostino^a, Giuseppe Lazzara^{a,b,*}, Francesco Ferrara^c^a Dipartimento di Fisica e Chimica, Università degli Studi di Palermo, Viale delle Scienze, pad. 17, Palermo 90128, Italy^b Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali, INSTM, Via G. Giusti, 9, Firenze 50121, Italy^c Department of Precision Medicine in Medical, Surgical and Critical Care, Università degli Studi di Palermo, Policlinico "Paolo Giaccone" Palermo - Edificio 4, Palermo 90128, Italy

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ABSTRACT

We investigated a fibrinogen–thrombin bicomponent formulation in 0.9% NaCl and reinforced with halloysite nanotubes (HNTs) at 0.7% and 1.4% (w/w). Oscillatory rheology (strain and time sweeps) shows progressive stiffening with HNTs: the storage modulus increases from up to 681 Pa whilst $\tan \delta$ remains ≤ 0.21 , indicating solid-like behavior under wet conditions. The linear viscoelastic region (LVER) as well as large-deformation crossover showed HNTs content dependence. Fluorescence microscopy evidences fibrin clustering with HNTs, and uniform gel filling of nanotubes acting as anchoring sites, producing isotropic densification rather than aligned bundling. TGA confirms the presence of halloysite and thermal stabilization. Finally, 0.7% HNTs provides a balanced increase in stiffness and LVER; 1.4% HNTs maximizes stiffness but reduces large-strain tolerance. These findings demonstrate that halloysite nanotubes effectively tailor the rheological and microstructural properties of fibrin-based composites. The present work establishes a proof-of-concept for the use of halloysite nanotubes as nanostructured reinforcing additives in fibrin systems, while future biological, adhesion, and hemostatic studies will be required to assess their biomedical performance.

1. Introduction

Haemorrhage control during surgery or trauma requires sealants that can function under wet conditions, resist irrigation, and maintain adhesion to soft tissues while being biocompatible and biodegradable. Among available options, fibrin-based sealants, comprising fibrinogen and thrombin, are widely used clinically because they replicate the final step of the coagulation cascade, promoting rapid fibrin polymerization and clot formation [1–4]. These sealants provide excellent haemostatic performance and are resorbable, making them suitable for diverse surgical applications [5]. However, their mechanical integrity is often limited, particularly under physiological strain or fluid shear, which can compromise adhesion and durability. This limitation has motivated reinforcement strategies using particulate fillers or nanomaterials to enhance stiffness, toughness, and handling properties [6]. Additionally, concerns have been raised regarding potential adverse effects of some fibrin sealants, such as air bubble formation in the body [7].

To address these challenges, halloysite nanotubes (HNTs) have emerged as promising candidates for composite biomaterials. HNTs are naturally occurring clays with a tubular morphology, hollow lumen, diameters of 40–80 nm, and lengths of 400–1000 nm [8]. Their unique

surface chemistry, composed by external siloxane and internal aluminol groups, enables hydrogen bonding and electrostatic interactions with proteins and polymers [9–12]. HNTs exhibit low cytotoxicity, biodegradability, high aspect ratio, mechanical strength, and large surface area, making them versatile for biomedical applications [13–17]. Their lumen allows encapsulation of drugs or bioactive molecules for controlled release, while their surfaces can be functionalized for targeted delivery [18].

Beyond drug and gene delivery, HNTs have been incorporated into polymeric scaffolds for tissue engineering, where they improve mechanical properties, crystallinity, and bioactivity, particularly in bone regeneration and wound healing contexts [8,13]. Recent studies report that HNTs-reinforced biopolymers exhibit enhanced tensile strength, reduced degradation rates, and improved cell adhesion and proliferation, supporting their role in regenerative medicine [16]. Furthermore, HNTs composites are being explored for biosensing, imaging, and enzyme delivery, expanding their biomedical relevance [19,20]. Their innate haemostatic potential and ability to interact with fibrin networks align well with the design of advanced tissue sealants. These attributes make HNTs attractive for integration into fibrin matrices, where they can reinforce the network mechanically and potentially modulate

* Corresponding author at: Dipartimento di Fisica e Chimica, Università degli Studi di Palermo, Viale delle Scienze, pad. 17, Palermo 90128, Italy.

E-mail address: giuseppe.lazzara@unipa.it (G. Lazzara).

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biological interactions.

In this study, we evaluated the effects of incorporating 0.7% and 1.4% (w/w) HNTs into a fibrinogen–thrombin sealant in isotonic saline. Oscillatory rheology quantified small- and large-strain mechanical behaviour (G' , G'' , $\tan \delta$, LVER, crossover), while fluorescence microscopy and thermogravimetric analysis (TGA) characterized microstructure and thermal signatures. Our goal was to correlate enhanced network mechanics with fibrin clustering and HNTs–protein interactions under physiologic conditions, guiding the design of fibrin-based nanocomposites.

2. Materials and methods

2.1. Materials

NaCl 0.9% physiological solution was given by Eurospital. Halloysite nanotubes (HNTs) are from New Zealand deposit and provided by Imerys Ceramics [21]. The HNTs purification was carried out by sedimentation method as reported elsewhere [22]. We purchased human-derived fibrinogen and thrombin, synthetic aprotinin, and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ from Sigma-Aldrich.

2.2. Methods

Rheological investigations were performed using a Discovery HR–1 rheometer (TA Instruments) equipped with a Parallel Plate X-Hatched geometry (40 mm diameter). Oscillation amplitude tests were conducted by applying a strain ranging from 0.1 to 100,000% at a constant angular frequency of $10 \text{ rad} \cdot \text{s}^{-1}$. In addition, oscillation time measurements were performed to evaluate the viscoelastic properties by determining the storage (G') and loss (G'') moduli as functions of time (from 0 to 300 s), applying a constant strain of 10% at an angular frequency of $10 \text{ rad} \cdot \text{s}^{-1}$.

The morphology of the sealant gels was examined by means of a microscope ESEM FEI Quanta 200 F. Prior to analysis, the samples were dried and their surfaces were coated with a thin gold layer in argon using an Edwards Sputter Coater S150A to avoid charging under an electron beam.

Optical images were acquired using a NeXcope NE710 optical microscope (TiEsseLab) equipped with a digital camera (TiEsseLab

MiChrome 20). In addition, fluorescence images were obtained using an LED light source (NeXcope FL 900 C LED) with excitation and emission wavelengths of 330 and 385 nm, respectively. The quantitative image analysis was carried out using Fiji software investigating fiber diameter and fluorescence gel fraction. Measurements are derived from fluorescence-based segmentation, assuming fibrin brightness. The optical solid fraction is a threshold-based proxy for dense regions and should be interpreted cautiously due to contrast variability (Fig. 1).

Thermogravimetric analyses (TGA) were carried out using a TGA550 apparatus (TA Instruments) under inert atmosphere, with nitrogen flow rates of 60 and $40 \text{ cm}^3 \cdot \text{min}^{-1}$ for the sample and the balance, respectively. Samples were heated in a platinum pan from 25 to 800°C with a heating rate of $20^\circ\text{C} \cdot \text{min}^{-1}$.

2.3. Samples preparation

Fibrinogen (45 mg cm^{-3}) was prepared in the presence of aprotinin (45 mg cm^{-3}) in NaCl 0.9% solution while thrombin solutions (0.143 mg cm^{-3}) were prepared in the presence of CaCl_2 ($0.020 \text{ mmol cm}^{-3}$) in NaCl 0.9% solution, since calcium ions are known to speed up clot formation following the addition of thrombin to fibrinogen [23]. The two solutions were mixed in a 1:1 vol ratio with a NaCl 0.9% solution to simulate the dilution effect in a physiological fluid. Upon mixing a gel like structure was obtained.

The same protocol was used in the presence of variable amounts of Halloysite nanotubular clay (0.7 and 1.4 wt%) in the NaCl solution. In both cases the gel formation was not disturbed. The increase in the gel turbidity in the presence of HNTs indicates the diffusion of the nanotubes into the gel structure. Fig. 2 provides a photo of the obtained gels and a schematic representation of the preparation protocol. For all experimental investigations, 5 independent samples were prepared, and the standard deviation was calculated and provided as error bars.

3. Results

A transparent gel is obtained immediately after mixing, in a 1:1 vol ratio, the fibrinogen and thrombin solutions in physiologic NaCl solution. The presence of halloysite nanotubes does not alter the general gel forming attitude despite a milky gel is obtained as expected for the

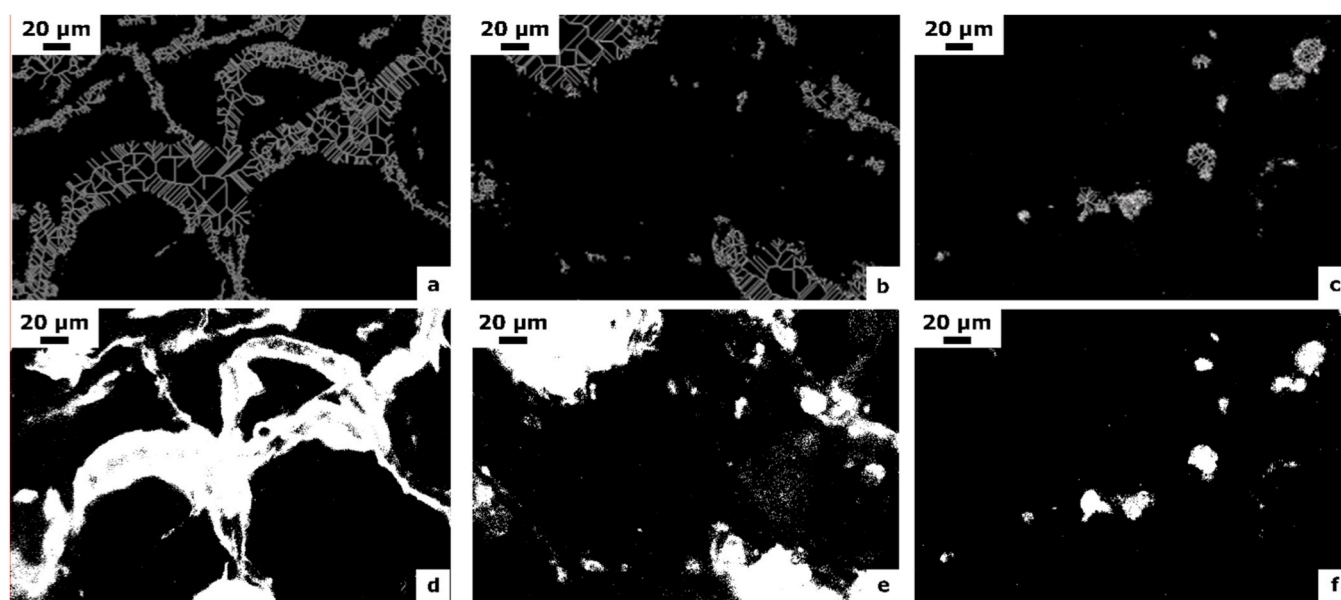


Fig. 1. Image analysis performed in Fiji-ImageJ software for fiber thickness and fluorescence area quantification. Top row (a–c): distance map segmentation used for local fiber thickness analysis; bottom row (d–f): threshold binary images used to quantify the fluorescent area fraction. HNTs concentration increases from left to right: (a, d) 0 wt%, (b, e) 0.7 wt%, and (c, f) 1.4 wt%.

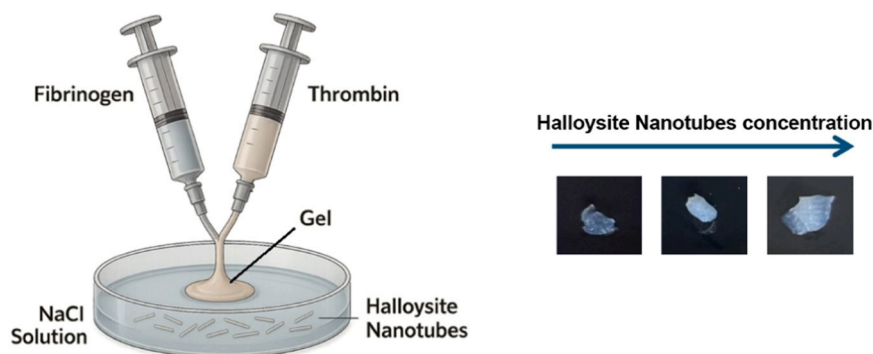


Fig. 2. Schematic presentation of the preparation protocol and optical photographs of the obtained gels.

incorporation of the nanoparticles into the gel matrices due to light scattering. It should be noted that the gelation occurs locally upon mixing without being negatively influenced by the dilution in the physiologic solution, and the halloysite nanotubes are efficiently incorporated into the high viscous gel. The physico-chemical characterization of the composite gels is provided in the following paragraphs.

3.1. Rheological analysis

Rheological tests were performed to evaluate the influence of halloysite nanotubes (HNTs) on the viscoelastic behavior of the sealant: oscillation amplitude and oscillation time measurements.

In oscillation amplitude tests a crossover point between the storage and loss moduli is clearly evidenced in all the analyzed samples (Fig. 3). Interestingly, it shifts toward lower strain values as the halloysite nanotube (HNTs) concentration increases, with the most pronounced effect observed at the highest HNTs loading. This trend demonstrates that, as the HNTs concentration increases, the transition from elastic to viscous behavior takes place at progressively lower strain values. Namely, smaller deformations are needed to favor the sealant flow when more nanotubes are incorporated.

On the other hand, oscillation time measurements showed that storage (G') and loss (G'') moduli remain essentially constant over the

entire range analyzed, indicating good stability over time (data are in supporting information). Moreover, G' remains higher than G'' for all the samples, confirming a predominantly elastic (solid-like) response of the sealant. Furthermore, G' values increase with increasing HNTs concentration, demonstrating that the nanotubes effectively stiffen the material and enhance its elastic resilience.

This behavior is consistent with the oscillation amplitude results: although HNTs reinforce the fibrin network and increase its elastic and storage moduli in the linear regime, the enhanced rigidity also makes the structure more fragile under increased deformation, leading to an earlier elastic-viscous transition.

The time-averaged moduli, linear viscoelastic regime (LVER) and cross over strain obtained from the rheological experiments are listed in Table 1, while the storage modulus and crossover point vs HNTs content are provided in Fig. 4.

3.2. Morphological features from optical and fluorescence microscopies

Optical and fluorescence images were acquired for each sample to assess the morphological evolution of the fibrin network after HNTs addition (Fig. 5). In the fluorescence images, the bright green signals correspond to fibrin [24] as HNTs are non-fluorescent [25].

Both optical and fluorescence images showed a clearly defined

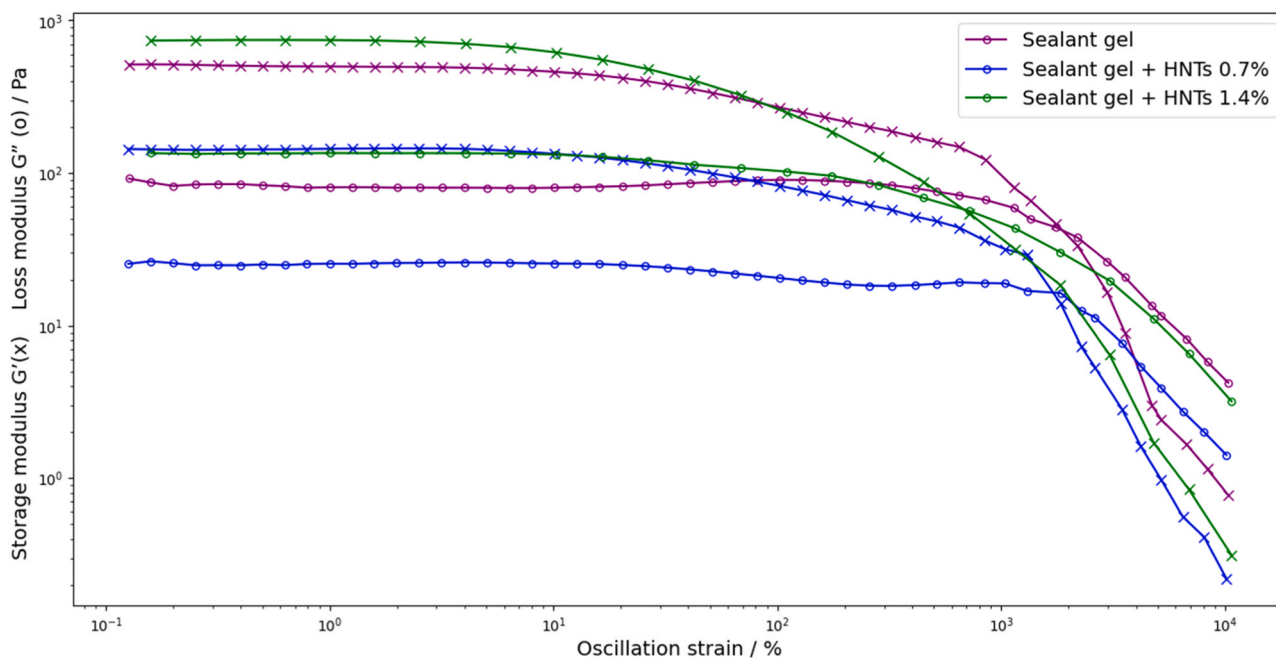


Fig. 3. Oscillation amplitude sweep at $\omega = 10 \text{ rad}\cdot\text{s}^{-1}$ for fibrin-based sealant and formulations containing halloysite nanotubes (HNTs) at 0.7% and 1.4% (w/w). Storage modulus (G') and loss modulus (G'') are plotted as a function of strain (γ , %) on a logarithmic scale.

Table 1
Rheological parameters.

Formulation	G' mean (Pa)	G'' mean (Pa)	tan δ mean	LVER end (% strain)	Crossover (% strain)
Sealant gel	386.6	68.4	0.177	5.03	2191
Sealant gel + HNTs 0.7%	541.1	109.5	0.203	10.08	1847
Sealant gel + HNTs 1.4%	681.0	142.3	0.209	4.03	723

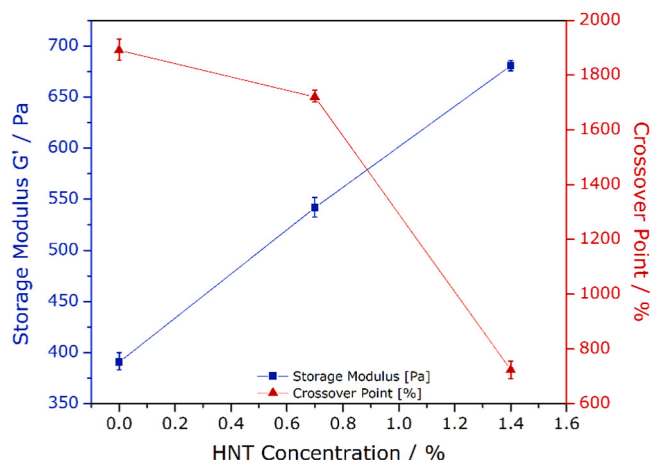


Fig. 4. Storage modulus and crossover point vs HNTs content.

fibrous matrix in the gel sealant. The increase of HNTs concentration results in a marked reduction in fluorescence intensity and a progressive loss of the fibrous organization of the protein. At the highest nanotube content, the structure is no longer dominated by fiber but instead shows the presence of compact, irregular clusters with only sparse or poorly defined elongated features, suggesting a disrupted or less interconnected network.

Such morphological observations provide support for the rheological interpretation: the transition from a continuous fibrous network to a more clustered and discontinuous structure is consistent with the reduced strain-bearing capacity and earlier deviation from elastic behavior detected in the oscillation amplitude tests at higher HNTs contents.

3.3. Characterization of the dried sealant

To evaluate the thermal effect of nanoclay incorporation on a fibrin-based sealant, thermogravimetric analyses (TGA) were performed. Additionally, the morphology of the xerogel was imaged by electron microscopy. Before measurements, samples were kept at 50°C to eliminate excess of water.

The thermogravimetric curves of the sealant (Figure S1) exhibited two distinct degradation steps of the protein as evidenced by the mass losses at 317.9 and 558.2 °C. Upon addition of halloysite nanotubes, the first degradation step remains unaffected, indicating that the initial thermal decomposition pathway, likely associated with peptide backbone degradation, is not influenced by the presence of nanotubes. Conversely, the second degradation step is shifted to higher temperatures. This shift suggests an enhancement in the thermal stability of the system, which can be attributed to the barrier effect and heat shielding properties of the nanotubes. In addition, interfacial interactions between fibrin and halloysite nanotubes may contribute to the observed stabilization. The charged surface of halloysite is known to interact with proteins through electrostatic interactions and hydrogen bonding,

leading to reduced chain mobility [26]. This restricted mobility can delay the thermal decomposition of the protein matrix, which is reflected in the shift of the second degradation step toward higher temperatures in the thermogram.

The scanning electron microscopy allowed us to image the nanotubes dispersed into the polymeric matrix (Fig. 6). The absence of preferential directions and long range order agrees with the optical microscopy findings. A uniform random dispersion of HNTs was observed demonstrating that the diffusion of the nanotubes occurred within the full volume of the sealant.

4. Discussion

The addition of halloysite nanotubes (HNTs) modulates fibrin-based sealant microstructure in a dose-dependent manner. Halloysite nanotubes effectively reinforce the fibrinogen–thrombin sealant in physiologic saline, increasing G' while maintaining solid-like behavior. For intraoperative use under irrigation, the observed G' range (≈ 400 – 700 Pa) and low $\tan \delta$ (≈ 0.18 – 0.21) are consistent with elastic sealing. The 0.7% HNTs composition offers improved handling (extended LVER) and stiffness. The 1.4% HNTs composition provides maximal stiffness, but it is less forgiving under large deformations (short LVER, low crossover). Microscopy reveals HNTs-induced fibrin clustering, likely mediated by ionic screening and protein–surface adsorption, consistent with observed mechanical changes.

The concept of wet-field haemostasis is particularly relevant for abdominal and gastrointestinal surgery, where sealants are routinely applied to actively bleeding or oozing tissue surfaces under persistent moisture and fluid flow [27–30]. In these settings, fibrin-based sealants are delivered topically as thin layers and undergo rapid in situ polymerization in the presence of blood, serous fluids or irrigation, forming a provisional fibrin barrier that must adhere to soft, compliant tissues while resisting washout. From a mechanical standpoint, gastrointestinal and abdominal applications are characterized by limited macroscopic tissue deformation combined with significant fluid shear, diffuse bleeding, and local pressure gradients, as encountered in parenchymatous organs or along serosal and anastomotic surfaces. Under such conditions, sealant performance depends on the ability to maintain cohesive integrity and surface conformity in a fully hydrated environment, rather than on tolerance to large cyclic strains [31,32]. Within this context, the mechanical reinforcement induced by HNTs highlights a trade-off between stiffness enhancement and deformability that directly impacts wet-field performance. Increasing elastic modulus can improve resistance to fluid-induced disruption and support effective sealing of oozing surfaces, while excessive loss of deformability may reduce the material's ability to adapt to irregular tissue topography. Accordingly, the observed balance between rigidity and compliance emerges as a key parameter for optimizing fibrin-based sealants intended for abdominal and gastrointestinal wet-field haemostasis. The observed increase in elastic modulus suggests that HNTs substantially modify the mechanical response of the fibrin network. Whether these rheological changes translate into improved adhesion, washout resistance or hemostatic performance requires dedicated functional investigations that are beyond the scope of the present study.

General trends with HNTs addition to fibrin gels include potential network densification, changes in fiber bundling due to templating/bridging, and altered pore size distribution. The quantitative image analysis capture these effects via fiber diameter and fluorescence gel fraction (Fig. 7).

The quantitative and morphological analysis reveals clear structural transitions across the three samples. The pure gel forms a connected yet heterogeneous network with the highest fiber thickness (median 5.6 μm) and a modest fluorescence gel fraction (34.72%), indicating the anisotropic architecture. Introducing 0.7% HNTs the fiber thickness decreases (mean 3.03 μm), together with the fluorescence fraction (26.26%), suggesting nanotube-driven bundling and partial directional

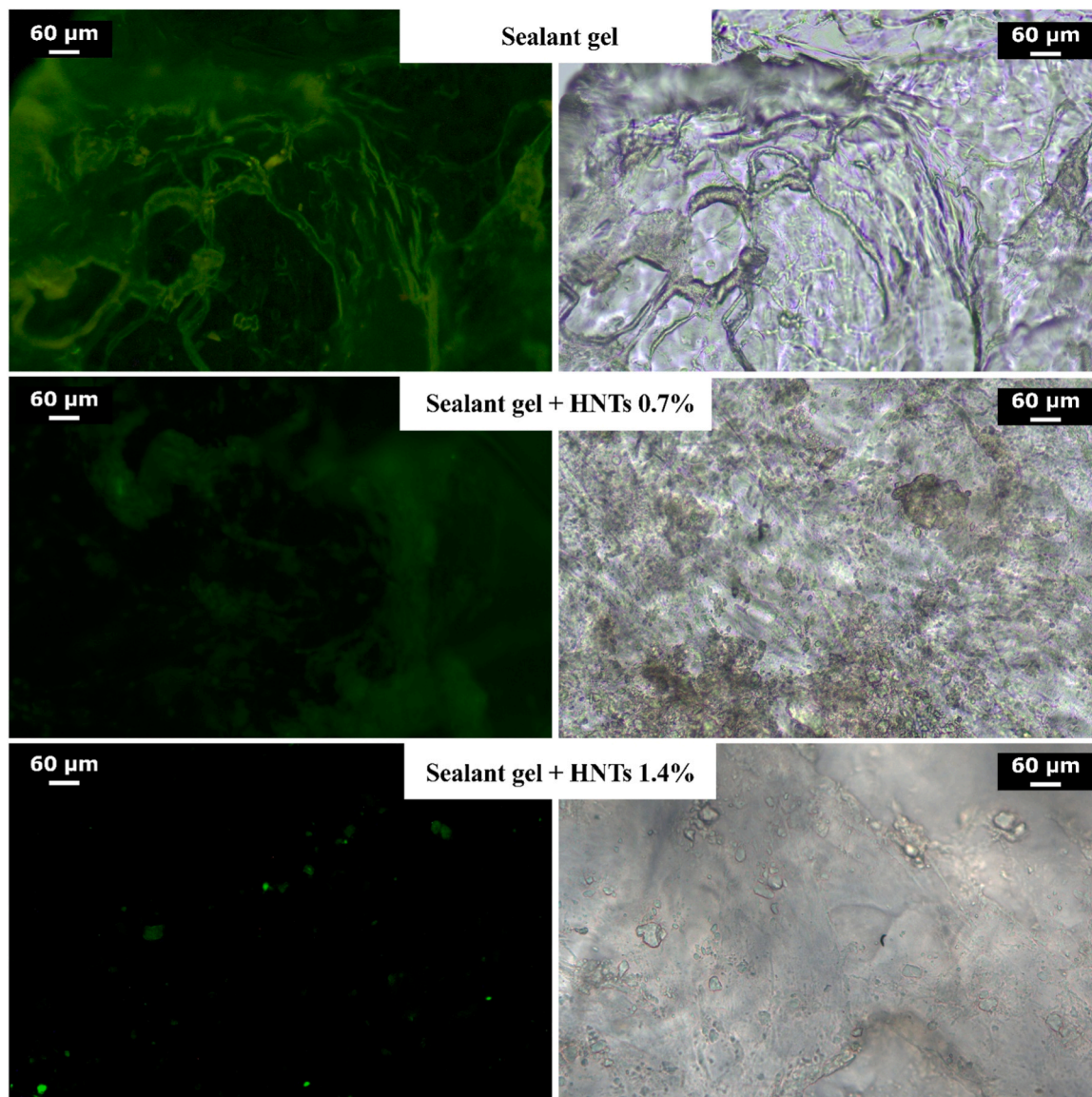


Fig. 5. Left hand side: Fluorescence images for sealant gel, sealant gel + HNTs 0.7%, sealant gel + HNTs 1.4%. Right hand side: the corresponding optical images.

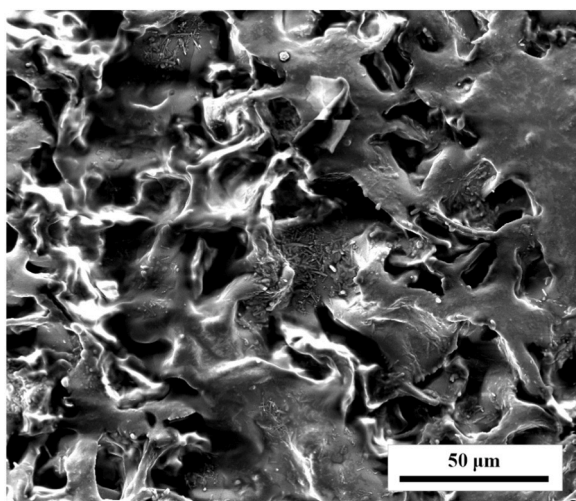


Fig. 6. Scanning electron microscopy image of sealant gel + HNTs 0.7%.

organization. At 1.4% HNTs, fiber is markedly reduced (mean 0.18 μm), as well as the fluorescent fraction (mean 1.4%), indicating a dense yet isotropic network. It should be noted that the observed decrease in fluorescence solid fraction at higher HNT loadings may not only reflect a reduction in fibrin network density but could also arise from fluorescence quenching effects induced by the presence of HNTs. Overall, increasing HNTs concentration shifts the gel from a heterogeneous anisotropic structure to a partially aligned, compact network at 0.7%, and finally to a uniformly densified isotropic network at 1.4%, highlighting the concentration-dependent role of HNTs in modulating fiber organization and pore architecture. The transition from an anisotropic to a more isotropic network may influence biological performance, potentially affecting cell infiltration and clot remodeling. While anisotropy can facilitate directional migration and remodeling, isotropic structures may provide a more uniform but less permissive environment for cell organization [33,34].

These findings align with the interfacial chemistry and morphology of HNTs, whose aluminol (inner) and siloxane (outer) surfaces, lumen, and high aspect ratio favor adsorption and reinforcement of biopolymers while maintaining low toxicity and broad biocompatibility [11]. Our observation that 0.7% HNTs promotes

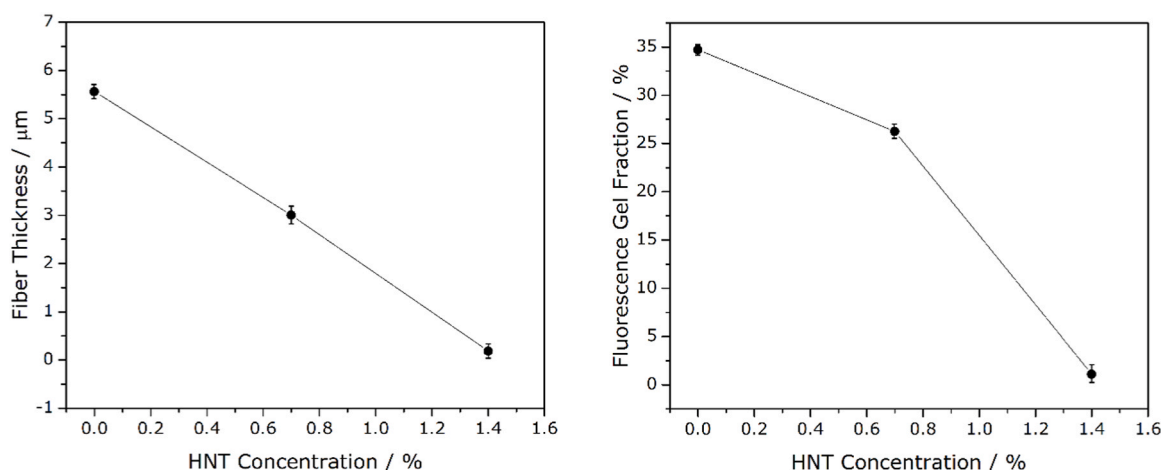


Fig. 7. Quantitative analyses to obtain fiber diameter and fluorescence solid fraction in the sealant as a function of the HNTs content. Data are obtained from fluorescence segmentation and threshold images.

bundle formation and partial alignment, whereas 1.4% HNTs yields isotropic densification, is also consistent with nanofiller–fibrin composite behavior reported for other colloidal fibers (e.g., cellulose nanocrystals), where filler-induced modification of fibrillogenesis and enhanced storage moduli accompany changes in network architecture. [35,36]

Finally, fluorescence-based quantification requires caution: labeling strategies and fluorophore concentrations can bias fibrinolysis behavior and apparent intensity.

5. Conclusions

A loading of 0.7% HNTs yields the best balance of small-strain robustness (LVER $\sim 10\%$) and stiffness (~ 541 Pa), supporting reliable handling. Increasing HNTs to 1.4% maximizes stiffness (~ 681 Pa) but shortens the LVER ($\sim 4\%$) and lowers the large-strain crossover ($\sim 723\%$), implying reduced tolerance to extreme deformation. Advanced image-analysis approaches further emphasize the importance of objective structural metrics to correlate network morphology with clot properties. This dose–response mirrors trends in HNTs–polymer composites, where modest loadings maximize dispersion and reinforcement, while higher contents promote filler–filler networking and more homogeneous stiffening without pronounced orientation. The rheological findings, together with microscopy and investigations on dried composites, suggest that the different HNT concentrations provide distinct rheological responses, offering a strategy to tune the viscoelastic properties of fibrin composites according to future application requirements. Although direct evaluation of clotting time, blood coagulation index, clot stability, wet-tissue adhesion, burst pressure, washout resistance, degradation behaviour, cytocompatibility and in vivo performance will be necessary before any clinical application, the present study establishes the first proof-of-concept that halloysite nanotubes can successfully reinforce fibrin-based sealants while preserving gel formation and providing tunable rheological behaviour. These findings identify halloysite nanotubes as a promising nanostructured additive for the rational design of next-generation fibrin sealants.

CRediT authorship contribution statement

Martina Maria Calvino: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Giulia D’Agostino:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Giuseppe Lazzara:** Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Francesco Ferrara: Writing – review & editing, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.nxmte.2026.102977](https://doi.org/10.1016/j.nxmte.2026.102977).

Data availability

Data will be available upon request to the corresponding author.

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