Hydroxyapatite Combined with Graphene Hydrogels for Hemostat Applications: An In Vitro Analysis

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KEYWORDS
Hemostat, Graphene Hydrogel, Hydroxyapatite, Uncontrolled Bleeding, Trauma, Clotting Time

ABSTRACT
Background: Uncontrolled bleeding remains a significant cause of mortality in traumatic injuries and surgical procedures. Hemostatic materials play a crucial role in preventing excessive blood loss and ensuring effective wound healing. Graphene-based hydrogels, particularly when combined with hydroxyapatite (HAP), offer a promising approach for hemostatic applications due to their high porosity, rapid blood absorption, and bioactivity.
Aim: This study aims to fabricate and evaluate HAP-graphene hydrogel membranes as potential hemostatic agents by assessing their clotting efficiency and morphological characteristics.
Materials and Methods: Graphite oxide (GO) was synthesized using the Hummers method. HAP was prepared using calcium carbonate and ammonium hydrogen phosphate under a calcination process. A composite HAP-GO membrane was fabricated by blending HAP and GO in a 1:1 ratio with alginate, followed by cross-linking with calcium chloride. The hydrogels were characterized using Scanning Electron Microscopy (SEM) for surface morphology analysis, and their hemostatic performance was evaluated by measuring clotting time.
Results: SEM analysis revealed a highly porous structure in the HAP-GO hydrogels, facilitating rapid blood absorption and interaction with clotting factors. The clotting time using the HAP-GO hydrogel was significantly reduced to 4 minutes and 10 seconds, compared to the normal clotting time of 5–7 minutes. The improved hemostatic efficiency was attributed to calcium ion release from hydroxyapatite, which accelerates the coagulation process.
Conclusion: The results indicate that HAP-GO hydrogels exhibit enhanced hemostatic properties, making them promising candidates for trauma care and surgical applications. Future research should focus on optimizing mechanical properties and biocompatibility for clinical translation..

1. Introduction:

Hemorrhage is a major cause of mortality in trauma and surgical settings, necessitating the development of effective hemostatic materials to minimize blood loss and promote wound healing (1). Traditional hemostatic agents, such as gauze and bandages, have limitations in clotting efficiency and adaptability to different wound types (2). Hydrogels, owing to their biocompatibility and tunable physical properties, have gained attention for hemostatic applications (3).

Graphene-based hydrogels, particularly those integrated with hydroxyapatite (HAP), have shown promise in hemostasis due to their superior mechanical stability, high porosity, and ability to facilitate platelet aggregation (4). Graphene-based sponges are novel hemostatic materials that exhibit fast fluid absorption, activation of clotting pathways, and structural versatility in different forms such as foams, membranes, and films (5).

Hydroxyapatite, a calcium phosphate mineral naturally found in bone and teeth, has been widely explored for biomedical applications due to its osteoconductive and bioactive properties (6). The incorporation of HAP into graphene hydrogels can enhance hemostasis by releasing calcium ions, which accelerate blood coagulation and improve wound healing (7).

This study focuses on the fabrication and in vitro evaluation of HAP-GO hydrogels for hemostatic applications, assessing their clotting efficiency and structural characteristics.

2. Materials and Methods

2.1. Synthesis of Graphite Oxide (GO) – Hummers Method

1. Graphite oxidation: Graphite powder and sodium nitrate were added to concentrated sulfuric acid

under an ice bath.

2. Addition of oxidant: Potassium permanganate was slowly introduced while stirring continuously.

3. Dilution and purification: The mixture was diluted with water, followed by hydrogen peroxide (H_2O_2) addition to complete oxidation.

4. Filtration and washing: The resulting graphite oxide (GO) was separated by filtration and thoroughly washed.

2.2. Synthesis of Hydroxyapatite (HAP)

1. Calcium carbonate (800 mg) was dissolved in 50 mL of distilled water.

2. 5 mL of diammonium hydrogen phosphate was added, forming a white precipitate.

3. The precipitate was dried at 80°C in a hot air oven, followed by calcination at 600°C for 3 hours to obtain HAP in powder form.

2.3. Fabrication of HAP-GO Hydrogel Membrane

1. HAP and GO (1:1 ratio) were blended with 10 mL alginate and stirred for 1 hour.

2. The mixture was freeze-dried at 4°C for 12 hours.

3. The resulting membrane was cross-linked with 6% calcium chloride for 30 minutes to enhance mechanical stability.

2.4. Characterization Techniques

Scanning Electron Microscopy (SEM): Used to examine the morphology and porosity of the hydrogel.

2.5. Hemostatic Evaluation

The clotting time was assessed by comparing untreated blood samples with those treated with the HAP-GO hydrogel.

3. Results

1. Clotting time analysis: Normal clotting time: 5–7 minutes Clotting time with HAP-GO hydrogel: 4 minutes and 10 seconds (Figure 1 & 2)

2. SEM analysis: (Figure 3 & 4)

The hydrogel exhibited a highly porous structure, promoting rapid blood absorption and interaction with platelets. Uniform distribution of HAP and GO nanoparticles was observed, indicating successful integration into the hydrogel matrix.

Figure 1: Pictorial representation of clotting without membrane placed at 4mins

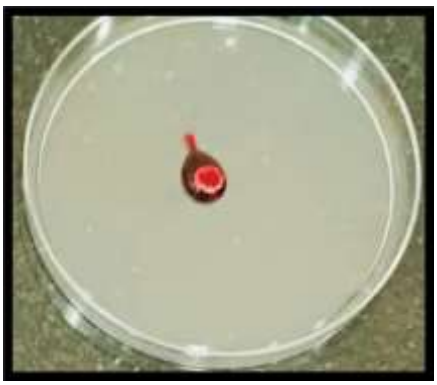

Figure 2: Pictorial representation of clotting with membrane placed at 4 mins

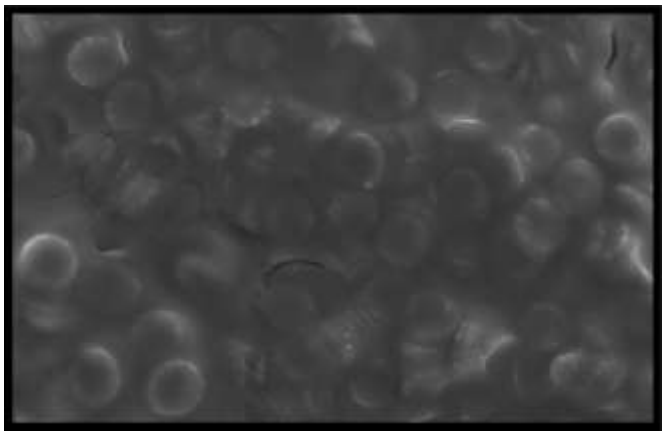

Figure 3: SEM Analysis of RBC of control group

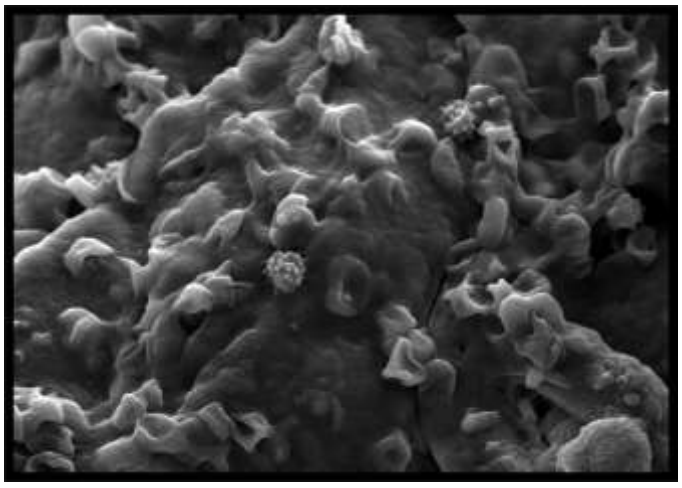

Figure 4: SEM analysis of RBC of treated material

4. Discussion

The HAP-GO hydrogel demonstrated superior hemostatic performance compared to natural clotting, reducing clotting time by approximately 20%. The release of calcium ions from hydroxyapatite accelerates coagulation, facilitating platelet aggregation and fibrin formation (8).

Graphene-based sponges have been widely studied for their rapid fluid absorption and activation of clotting pathways (9). The porous structure of the hydrogel enhances oxygen exchange at the wound site, which is essential for tissue regeneration and healing (10).

In addition to hemostatic efficiency, biocompatibility remains a critical factor. Both HAP and GO have demonstrated low cytotoxicity in previous studies (11). However, long-term in vivo studies are

required to evaluate potential immunogenic responses and degradation profiles (12).

Graphene-based materials also offer potential for multifunctional applications, such as drug delivery and tissue engineering. Future studies should explore composite hydrogels incorporating bioactive molecules or clotting factors for enhanced therapeutic effects (13).

5. Conclusion

The HAP-GO hydrogel membrane demonstrated improved hemostatic performance, reducing clotting time and enhancing platelet interactions. The porous structure and calcium ion release play a crucial role in accelerating clot formation. Given its biocompatibility and mechanical stability, this material holds promise for trauma care and surgical applications. Further research is required to optimize biodegradability, mechanical properties, and in vivo functionality.

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