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Magnetic hydrogels functionalized with *Divi-Divi* extract for tissue engineering

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Abstract: Magnetic hydrogels are emerging as promising biomaterials for tissue engineering due to their ability to provide structural support and controlled drug release. In this study, gelatin-hyaluronic acid hydrogels (70:30) were synthesized and functionalized with *Divi-Divi* (Libidibia coriaria) extract (1% w/v) and magnetic nanoparticles (Fe_3O_4@SiO_2, 1% w/v). The extract was obtained through an aqueous extraction method and characterized for its bioactive properties. Rheological analysis showed that the incorporation of magnetic nanoparticles increased hydrogel stiffness, whereas Divi-divi extract did not significantly affect the mechanical properties. Controlled release experiments were conducted using a 30 mT magnetic field at 100 kHz for 30 minutes in PBS, resulting in a release of approximately 62 µg/ml of the extract, as detected by UV spectrophotometry. These results demonstrate the potential of these hydrogels as magnetically responsive drug delivery systems for biomedical applications. Future studies will optimize the release kinetics and evaluate their biocompatibility in vitro.

Keywords: magnetic hydrogels, controlled released, natural antimicrobial extract, *Divi-Divi*, tissue engineering

1 Introduction

Tissue engineering is a rapidly growing field focused on the development of biomaterials that promote tissue regeneration. Hydrogels, particularly those based on natural biopolymers like gelatin and hyaluronic acid, have gained significant attention due to their excellent biocompatibility and ability to mimic the extracellular matrix [1]. Magnetic hydrogels, in particular, hold great potential in controlled drug delivery systems as they can be manipulated using external magnetic fields [2]. The incorporation of bioactive natural extracts, such as *Divi-Divi (Libidibia coriaria)*, offers additional benefits, including antimicrobial and anti-inflammatory effects, further enhancing their therapeutic potential [3].

This study aims to develop and characterize magnetic hydrogels functionalized with Divi-Divi extract for use in tissue engineering applications. The hydrogels were synthesized using a combination of gelatin, hyaluronic acid, magnetic nanoparticles (Fe_3O_4@SiO_2), and *Divi-Divi* extract. The objectives include evaluating the mechanical and rheological properties of the hydrogels and assessing their ability to release the natural extract under magnetic stimulation.

2 Materials and methods

2.1 Hydrogel synthesis

A 1% w/v solution of hyaluronic acid (HA) was prepared in deionized water, adjusted to pH 0.5 with 20 M HCl, and stirred at 200 rpm for 24 hours at 37°C. The pH was then raised to 7 using 5 M NaOH. The product was dialyzed for 3 days with a 3500 MWCO membrane against deionized water, with the water being changed three times a day, and finally lyophilized.

For the tyramine grafting into HA, a 0.5% HA solution in deionized water was prepared with NaCl (150 mM), MES (0.276 mM), and NaOH (5 M) at pH 5.75. The mixture was stirred at 500 rpm for 2 hours at room temperature. Then, HCl-tyramine (molar ratio Tyr/COOH 2:1) was added, and the solution was stirred for 20 minutes, adjusting the pH to 5.75. EDC (molar ratio EDC/COOH 1:1) and NHS (molar ratio NHS/EDC 1:10) were added, and the reaction was carried out for 24 hours at 37°C. The mixture was then dialyzed for 2 days using a 3500 MWCO membrane, first against 150 mM NaCl for 24 hours and then against deionized water for another 24 hours. Finally, the HA-Tyr sample was lyophilized.

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The gelatin (GEL) hydrogel was prepared by dissolving GEL at 2% w/v in 50 mM MES, stirring for 30 minutes at 60°C. Then, HCl-tyramine (molar ratio Tyr/COOH 2:1) was added, and the mixture was stirred for 20 minutes at room temperature. The pH was adjusted to 6, and NHS (molar ratio NHS/EDC 1:10) was added, followed by 30 minutes of stirring. EDC (molar ratio EDC/COOH 2:1) was then incorporated, and the reaction was carried out for 24 hours at 37°C. The resulting solution was dialyzed for 2 days using a 12,400 MWCO membrane, with deionized water being replaced three times a day, and the GEL-Tyr sample was lyophilized.

2.2 *Divi-Divi* synthesis

Divi-Divi was collected in the municipality of La Paz, Cesar, Colombia (coordinates: 10°23'18"N-73°11'55"W, 127 masl). Taxonomic identification was performed at the Colombian National Herbarium (COL 632490, 2024). The green fruits of *Divi-Divi* were freeze-dried and finally ground. The resulting powder was subjected to exhaustive solid-liquid extraction with sonication at room temperature. Type I water was used as a solvent. The aqueous plant extract (APE) obtained was concentrated by vacuum distillation at 50°C and 80 mbar. The concentrated APE was completely dried by freeze-drying (0.1 mbar and -55.7°C). The residual powder was stored at 4°C until use.

2.3 Magnetic nanoparticle synthesis

Magnetic nanoparticles (Fe_3O_4@SiO_2) were synthesized using a sol-gel method. Initially, a solution of iron(III) chloride ($FeCl_3$) was prepared by dissolving 4.814 g of $FeCl_3$ in 100 mL of deionized water under constant stirring. Simultaneously, iron(II) chloride ($FeCl_2$) was added to the solution, forming a mixture of iron salts. This solution was subjected to sonication for 10 minutes at a frequency of 70% and a power of 500 W to ensure better dispersion and uniform mixing of the salts. Following this, sodium hydroxide (NaOH) was added dropwise to adjust the pH, which caused the precipitation of magnetite (Fe_3O_4) nanoparticles.

To isolate the magnetite, a strong neodymium magnet was applied to the solution for 1 hour, allowing the magnetite particles to decant. The supernatant was discarded, leaving behind the magnetite particles, which were then washed twice with deionized water to remove residual salts and impurities.

For the silica coating, the magnetite particles were dispersed in 60 mL of ethanol. This dispersion was sonicated for 10 minutes under the same sonication conditions, maintaining a maximum temperature of 40°C. Tetraethyl orthosilicate (TEOS) was then added to the solution (500 µL) while continuing the sonication process. Afterward, ammonia solution (NH_3) was introduced to catalyze the hydrolysis and condensation of TEOS, promoting the formation of silica around the magnetite nanoparticles. The reaction was allowed to continue for 1 hour under sonication at the same conditions.

Once the silica coating was completed, the magnetite-silica nanoparticles (Fe_3O_4@SiO_2) were separated from the solution using a neodymium magnet for 1 hour. The particles were washed five times with ethanol to remove any unreacted materials and excess solvent. Finally, the nanoparticles were dried at 60°C for 8 hours to ensure complete removal of the ethanol.

2.4 Magnetic hydrogel preparation

For the magnetic hydrogel preparation, HA and GEL were dissolved at 1% w/v in a 70:30 ratio in normal saline. A corresponding volume of 10% v/v horseradish peroxidase (HRP) (12.5 U/mL) and *Divi-Divi* extract was added to reach a final concentration of 1 mg/mL. Magnetic nanoparticles (Fe_3O4@SiO_2) at 1% w/v were then incorporated and mixed for 5 minutes using ultrasound. After the crosslinking was performed by adding 10% v/v H_2O_2, the crosslinking process was maintained for 20 minutes at room temperature.

2.5 Characterization

2.5.1 Chemical composition

The chemical composition was determined using FTIR Spectroscopy analysis (Perkin Elmer Spectrum 100, USA). The materials' FTIR spectrum was recorded in the mid-infrared region (4000–650 cm−1 wavenumber range) with a resolution of 1 cm−1.

2.5.2 Rheological properties

Dynamic properties were measured using a rheometer (Kinexus Ultra plus, NETZSCH GmbH, Germany) with a plate-plate configuration with sandblasted surfaces (diameter: 25 mm). A solvent trap was employed to avoid sample drying. A gap size of 1 mm was used. All measurements were performed at 37°C. The monomers were mixed with HRP and then added to the lower plate. The H2O2 was added to start the cross-linking process directly before the upper plate was

lowered. The hydrogel was kept in a steady state for 20 min before measurement to ensure complete gelation.

An amplitude sweep sequence with an oscillatory strain from 0.1 to 100 % (from 0.1 to 100% shear strain) at a frequency of 1 Hz was conducted to assess the linear viscoelastic region as well as the storage (G') and loss modulus (G'').

2.5.3 Controlled release

For release measurements, 1 mL of magnetic hydrogels with *Divi-Divi* extract was mixed with 1 mL PBS in 2 mL vials and placed in a MagneTherm RC coil (Nanotherics) at 30 mT and 100 kHz for 30 minutes. Samples were then centrifuged at 1000 rpm for 10 minutes, and the extract concentration in the supernatant was measured using UV spectrophotometry (Libra S22, Biocrom) with a calibration curve. A non-stimulated sample served as a control.

3 Results

3.1.1 Chemical composition

The FTIR spectrum of the magnetic hydrogels functionalized with *Divi-Divi* is presented in Figure 1. B with a peak at 1630 cm^{-1} corresponding to the amide I band, characteristic of the peptide bonds in GEL. Additionally, peaks at 1600 cm^{-1} and 1400 cm^{-1} were observed, attributable to the stretching vibrations of the carboxyl group and C-H bonds of HA. The *Divi-Divi* extract presented bands at 1600 cm^{-1} and 1100 cm^{-1}, indicating the presence of polyphenolic compounds.

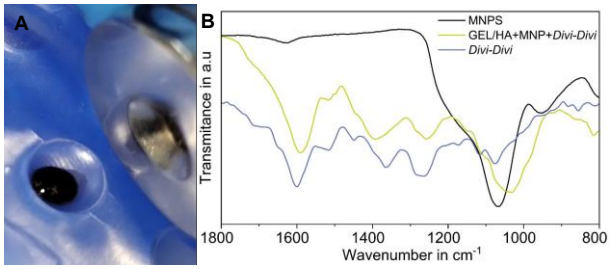

Figure 1: Magnetic hydrogel (A) and FTIR spectra (B).

The silica-coated magnetite nanoparticles showed a peak at 1080 cm^{-1}, corresponding to the Si-O stretching, confirming the silica coating on the magnetite particles.

Overall, the spectrum of the final magnetic hydrogel showed no significant shifts in the functional groups, suggesting that the incorporation of magnetic nanoparticles and the extract did not alter the chemical structure of the hydrogel matrix.

3.1.2 Rheological properties

The rheological analysis of the linear viscoelastic region demonstrated that the incorporation of magnetic nanoparticles into the hydrogel significantly increased the mechanical strength. The storage modulus (G') of the magnetic hydrogels, as shown in Figure 2, was measured at 239.6 Pa, compared to 89.18 Pa for the hydrogel without nanoparticles, indicating a significant increase in stiffness. Additionally, the incorporation of magnetic nanoparticles increased the viscoelastic region. In contrast, the addition of *Divi-Divi* extract at 1 mg/mL had no significant effect on the elastic properties, with G' remaining at approximately 200 Pa.

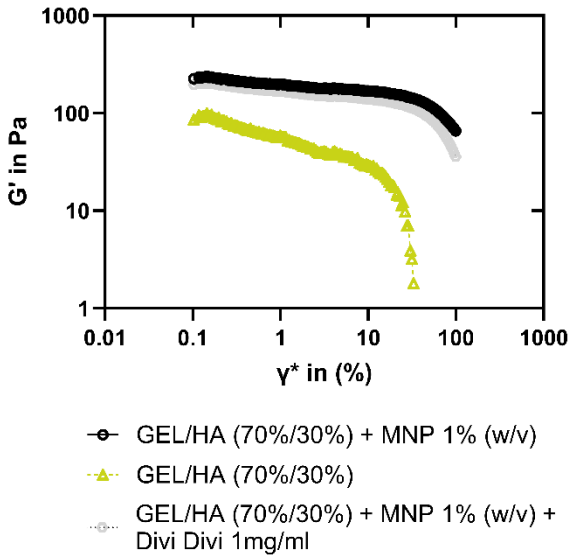

Figure 2: Storage modulus as a function of oscillatory strain. The incorporation of magnetic nanoparticles (MNPs increased the storage modulus. However, the addition of Divi-Divi extract had no impact on the dynamic elastic properties.

3.1.3 Controlled release

The release profile of the magnetic hydrogels functionalized with *Divi-Divi* extract showed a significant increase under magnetic stimulation compared to the non-stimulated control. In the absence of magnetic field stimulation, the release of *Divi-Divi* extract was measured at 30 µg/ml, whereas the magnetically stimulated samples released approximately 62 µg/ml of the extract. This marked difference in release suggests that the application of the magnetic field plays a key role in enhancing the controlled release of the bioactive compound from the hydrogel matrix.

4 Discussion

The results of this study demonstrate the promising potential of magnetic hydrogels functionalized with *Divi-Divi* extract for tissue engineering applications, particularly in controlled drug delivery. The chemical characterization confirmed the successful incorporation of both the natural *Divi-Divi* extract and magnetic nanoparticles within the hydrogel matrix. The FTIR spectra showed distinct peaks corresponding to the functional groups of GEL, HA, and *Divi-Divi* extract, suggesting effective integration of the bioactive compound into the hydrogel. Additionally, the presence of Fe-O bonds, characteristic of the silica-coated magnetic nanoparticles, was identified, verifying their incorporation into the hydrogel matrix.

Oscillatory measurement revealed that the incorporation of magnetic nanoparticles increased the hydrogel stiffness, which is essential for tissue engineering applications where structural support is needed. It also increased the region's viscoelastic integrity, suggesting additional support of the polymer chains. However, the addition of *Divi-Divi* extract did not significantly alter the dynamic response of the hydrogel, indicating that the extract was well integrated without disrupting the mechanical behavior of the matrix, while keeping the improved viscoelastic response. The viscoelastic nature of the hydrogels supports their potential for mimicking the extracellular matrix, which is important for tissue regeneration.

The controlled release experiments showed the effectiveness of the magnetic hydrogels in drug delivery. The release of *Divi-Divi* extract from the hydrogels under magnetic stimulation was approximately 62 µg/ml, while the non-stimulated control released only 30 µg/ml. This significant difference in release shows the potential of using an external magnetic field to enhance the release of bioactive compounds from the hydrogel matrix. The magnetic nanoparticles within the hydrogel likely generate local heating effects or induce changes in the hydrogel structure, facilitating the release of the encapsulated extract. These results are in line with other studies that have demonstrated the ability of magnetic nanoparticles to control the release of drugs via external stimuli, confirming the potential of these hydrogels for controlled drug delivery applications [4].

5 Conclusions

The controlled release observed in this study demonstrates the potential of the developed magnetic hydrogels as a viable platform for targeted drug delivery. Although the release of the *Divi-Divi* extract was successfully triggered by a 30 mT magnetic field at 100 kHz, further optimization of the release kinetics is necessary to improve the precision and efficiency of drug release, and additional studies investigating the effect of varying magnetic field strengths, frequencies, and exposure times will help identify the most effective conditions for specific therapeutic applications.

The chemical and rheological properties demonstrated in this study suggest that these hydrogels possess the necessary characteristics for biomedical applications, including adequate mechanical strength and stability. Additionally, the incorporation of the *Divi-Divi* extract provides the hydrogels with antimicrobial and anti-inflammatory properties.

In conclusion, the development of these magnetic hydrogels functionalized with bioactive natural extracts represents a significant advancement in controlled drug delivery systems. These hydrogels offer an innovative solution for localized and targeted therapies, with great potential for applications in tissue repair and regeneration.

Author Statement

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Conflict of interest: The Authors state no conflict of interest.

References

[1] Saiz Culma JJ, Guevara Morales JM, Hata Uribe YA, Garzón-Alvarado DA, Leal-Marin S, Glasmacher B, Vaca-González JJ. Effects of electric fields on the modulation of chondrocytes dynamics in gelatin scaffolds. J Biomater Sci Polym Ed. 2025; Feb 25; 1-20.

[2] Liu Z, Liu J, Cui X, Wang X, Zhang L, Tang P. Recent Advances on Magnetic Sensitive Hydrogels in Tissue Engineering. Front Chem. 2020; 8:124.

[3] Pitre-Ruiz L, Galván-Ayala D, Castro-Uriana O, Ávila Mende K.J, Lopez-Pazos S.A. In vitro antimicrobial activity of Caesalpinia coriaria (Jacq.) Willd extracts on Streptococcus pyogenes and Candida albicans. Vitae. 2022; 28(2); e5.

[4] Liu JF, Jang B, Issadore D, Tsourkas A. Use of magnetic fields and nanoparticles to trigger drug release and improve tumor targeting. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2019 Nov;11(6):e1571.