Development and Characterization of a Citric Acid-crosslinked Cassava Starch Hydrogel Functionalized with Aloe Vera for Potential Tissue Repair Applications

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Abstract *— This study presents the development and characterization of a bio-based hydrogel synthesized from cassava starch and citric acid through a green approach and functionalized with Aloe vera. The material was prepared using a two-stage thermal curing process (110 °C for 1 h followed by 140 °C for 30 min) and evaluated for physicochemical, electrical, and antibacterial properties. FTIR-ATR analysis indicated the formation of ester linkages associated with starch–citric acid crosslinking. Swelling analysis revealed limited aqueous stability, with several formulations showing mass loss rather than consistent swelling. Surface pH values ranged from 2.02 to 3.55, indicating a strongly acidic profile associated with residual citric acid. Electrical characterization showed a formulation-dependent response related to ionic transport within the hydrogel matrix. A qualitative reduction in Escherichia coli growth was observed; however, quantitative antibacterial analysis was not performed. Overall, the results demonstrate the influence of formulation composition on hydrogel behavior and highlight the need for further optimization and biological validation.*

Keywords *— Aloe Vera, Cassava Starch, Citric Acid, Impedance Spectroscopy.*

INTRODUCTION

The field of regenerative medicine is an exciting area of research that aims to restore or replace damaged tissue by creating biomimetic materials [1]. Hydrogels are biomimetic materials that possess desirable characteristics, such as high-water content, biocompatibility, and structural similarity to the extracellular matrix (ECM) [2], [3]. Due to these properties, hydrogels can be used in various biomedical-related applications, including drug delivery systems and biofunctional materials [4].

Recently, there has been increased interest in developing environmentally friendly bio-based hydrogels derived from natural polymers [5]. Starch-based materials are inexpensive, biodegradable, and widely available [6], but their use may be limited by poor mechanical stability and rapid degradation in aqueous environments [7]. Chemical modification techniques, such as crosslinking with organic acids, together with the incorporation of natural bioactive components such as *Aloe vera*, have been investigated to improve the physicochemical and functional properties of these materials [8], [9].

PROBLEM STATEMENT

While increasing attention has been directed toward the use of bio-derived hydrogels for biomedical applications, the influence of formulation variables on their multifunctional behavior remains insufficiently understood [5], [10]. In particular, the combined effects of citric acid crosslinking and *Aloe vera* incorporation on the physicochemical, electrical, and antibacterial properties of starch-based hydrogel systems have not been systematically investigated [11], [12]. Additionally, the influence of bioactive component concentration on functional responses such as ionic conductance and antibacterial behavior remains poorly established [10], [13].

Therefore, the objective of this study was to develop and characterize cassava starch-based hydrogels with varying concentrations of citric acid and *Aloe vera* powder to establish relationships between formulation composition and material performance. The investigated properties included swelling behavior, surface pH, electrical conductance, and antibacterial response against *Escherichia coli* [4], [14]. This approach aims to provide insight into the design of sustainable hydrogel systems with tunable functional properties for bio-based material applications.

MATERIALS AND METHODS

Bio-based hydrogels composed of cassava starch, citric acid, and *Aloe vera* powder (AVP) were prepared using a formulation-based experimental approach to evaluate the influence of composition on the physicochemical, electrical, and antibacterial properties of the resulting materials [15]. Ten formulations (S1–S10) were synthesized by varying cassava starch concentration (5.0–7.0 g), citric acid content (0.5–0.7 g), and AVP incorporation (0.0–1.0 g) while maintaining a final solution volume of 100 mL distilled water for all samples (Table 1). All formulations were prepared and analyzed in triplicate. The hydrogel mixtures were prepared in 150 mL borosilicate glass beakers with an approximate internal diameter of 60 mm, which served as casting vessels during thermal curing.

Swelling behavior was evaluated using a gravimetric immersion method. The initial dry mass (W_0) of each hydrogel sample was recorded before immersion in distilled water at room temperature for 24 hours. After immersion, excess surface water was carefully removed, and the swollen mass (W_t) was measured. The swelling ratio (SR) was calculated according to (1).

$$SR\ (\%) = [(W_{(s)} - W_{(d)}) / W_{(d)}] \times 100 \quad (1)$$

Physicochemical characterization included pH measurements using a Mettler Toledo FiveEasy™ pH meter and Fourier-transform infrared (FT-IR) spectroscopy to evaluate chemical interactions and crosslinking within the hydrogel matrix. FT-IR spectra were collected using a PerkinElmer Spectrum Two FTIR Spectrometer in the spectral range of 4000–500 cm^{-1}. Morphological observations were performed using a Nikon Eclipse Ci-L optical microscope, while fluorescence microscopy analysis was conducted using a Nikon DS-Qi2 fluorescence microscope.

Electrical characterization was performed using a Keysight Technologies E4990A impedance analyzer coupled with a Keysight Technologies 16451B Dielectric Test Fixture integrated with the Keysight Materials Measurement Suite. Semi-solid hydrogel samples with circular disk-like geometry were analyzed between parallel circular electrodes under constant contact conditions. Impedance and conductance measurements were obtained to evaluate ionic transport behavior within the polymeric matrix. Electrical conductivity was determined according to (2), where f corresponds to the applied frequency, ε_0 is the vacuum permittivity, and ε'' represents the dielectric loss factor of the material.

$$\sigma = 2\pi f \varepsilon_0 \varepsilon'' \quad (2)$$

Finally, antibacterial behavior was qualitatively assessed using the Kirby–Bauer disk diffusion method against *Escherichia coli* by observing the presence or absence of visible bacterial growth reduction surrounding the hydrogel samples after incubation.

Materials

Cassava starch (*Manihot esculenta*) was used as the primary biopolymer matrix. Anhydrous citric acid ($C_6H_8O_7$, ≥99.5%) served as the crosslinking agent, while *Aloe vera* powder (AVP) was incorporated as the bioactive component. Distilled water was used as the solvent for all formulations, and 70% isopropyl alcohol was used for sterilization procedures. *Escherichia coli* was selected as the model microorganism for antibacterial evaluation.

Synthesis of the Bio-based Hydrogel

The hydrogels were synthesized via thermally induced esterification [16]. Cassava starch (*Manihot esculenta*) was dispersed in 100 mL of distilled water and heated to 80 °C under constant stirring (500 rpm) for 45–60 minutes until complete gelatinization was achieved. Anhydrous citric acid was then incorporated as the crosslinking agent, followed by the addition of *Aloe vera* powder at varying concentrations [17]. The mixture was further stirred to ensure homogeneous distribution of all components.

The resulting pre-gel solution was subjected to solvent evaporation and subsequently cured through a two-stage thermal process to promote esterification between starch and citric acid. The samples were first heated to 110 °C for 1 hour to facilitate dehydration, followed by heating to 140 °C for 30 minutes to induce crosslinking [18]. The obtained hydrogels were then allowed to cool to room temperature and stored in a desiccator prior to characterization.

Experimental Design

The experimental design evaluated the combined effects of cassava starch concentration, citric acid content, and incorporation of *Aloe vera* powder (AVP) on the physicochemical, electrical, and antibacterial behavior of the synthesized starch-based hydrogel system [13], [19]. A total of 10 formulations (S1–S10) were developed by systematically varying formulation composition while maintaining consistent synthesis conditions throughout the study. All formulations were prepared and analyzed in triplicate to ensure reproducibility [14]. The composition of the hydrogel series is summarized in Table 1.

Table 1
Composition of Hydrogel Formulations (S1–S10)

Sample	Starch (g)	Citric Acid (g)	*Aloe vera* (g)
S1	5.0	0.5	0.0
S2	5.0	0.5	0.2
S3	5.0	0.5	0.5
S4	5.0	0.5	1.0
S5	5.0	0.7	0.5
S6	5.0	0.7	1.0
S7	7.0	0.5	0.5
S8	7.0	0.5	1.0
S9	7.0	0.7	0.5
S10	7.0	0.7	1.0

The synthesized hydrogels were characterized to evaluate their physicochemical, electrical, and antibacterial properties. A combination of analytical techniques was employed to assess the chemical structure, swelling behavior, surface pH, electrical response, and antibacterial behavior of the formulations [13], [19]. These characterization methods enabled the evaluation of the influence of composition on the overall behavior of the hydrogel system.

The chemical structure and formation of ester linkages were analyzed using Fourier Transform Infrared (FT-IR) spectroscopy [12], [15]. Swelling behavior was evaluated by immersing samples in distilled water and monitoring mass variation, while surface pH was measured using a digital pH meter. Electrical properties were assessed using Electrochemical Impedance Spectroscopy (EIS), providing information on impedance and the overall electrical response of the materials [4], [10].

Antibacterial activity against *Escherichia coli* was evaluated by visual observation of bacterial growth in a disk diffusion assay and further examined by microscopy using a Nikon Eclipse Ni microscope. This approach provided a qualitative assessment of bacterial distribution at the hydrogel interface.

FT-IR Characterization

Fourier transform infrared (FT-IR) spectroscopy was performed to analyze the chemical structure of the synthesized hydrogels and identify the functional groups present within the polymeric network [12], [15]. The spectra were recorded over a wavenumber range of 4000–500 cm^{-1} using an FT-IR spectrometer operating in attenuated total reflectance (ATR) mode. Prior to analysis, the hydrogel samples were dried to minimize interference from residual moisture. The obtained spectra were used to identify characteristic absorption bands associated with hydroxyl (–OH), carbonyl (C=O), and other functional groups relevant to the hydrogel system [5], [17].

Swelling Test Procedure

The swelling behavior of the hydrogels was evaluated to determine their response in an aqueous environment [5], [12]. Dried hydrogel samples were initially weighed (W_0) and then immersed in distilled water at room temperature. At predetermined time intervals, the samples were removed, gently blotted to remove excess surface water, and weighed again (W_t) [17]. The swelling ratio was calculated from the mass difference between the swollen and dry states, providing information on the polymeric network's hydrophilic nature and structural stability [15], [18].

pH Measurements

The surface pH of the hydrogel formulations was measured to evaluate their chemical environment. Each hydrogel sample was placed in contact with a defined volume of distilled water and allowed to equilibrate prior to measurement. The pH was then recorded using a calibrated digital pH meter. All measurements were conducted at room temperature, and each sample was analyzed in triplicate to ensure reproducibility.

Electrical Measurement Procedure

The electrical properties of the hydrogel formulations were evaluated using an LCR meter to assess their electrical response and ability to support charge transport. Measurements were performed over a frequency range from 20 Hz to 20 MHz. The hydrogel samples were placed between the electrodes, and the system recorded impedance and related electrical parameters as a function of frequency. From these measurements, electrical conductance and loss tangent (tan δ) were obtained through the instrument's integrated analysis. All measurements were conducted at room temperature under controlled conditions [10], [20].

Antibacterial Testing Procedure

The antibacterial activity of the hydrogel formulations was evaluated against *Escherichia coli* using a disk diffusion method. Bacterial cultures were prepared in Tryptic Soy Broth (TSB) by inoculating 9 mL of sterile broth with 500 µL of *E. coli* suspension and incubating under aerobic conditions to obtain an actively growing culture. The resulting suspension was then uniformly spread onto Tryptic Soy Agar (TSA) plates to form a confluent bacterial lawn.

Hydrogel samples were applied onto the surface of the inoculated agar under sterile conditions. The plates were incubated at 37 °C for 24 hours under aerobic conditions (Figure 1). Antibacterial response was assessed by visual inspection of bacterial growth surrounding the samples.

Microscopic observations were performed using a Nikon Eclipse Ci-L optical microscope, and images were captured to evaluate bacterial distribution at the hydrogel–bacteria interface. This qualitative approach is commonly used in preliminary assessments of antibacterial behavior in hydrogel-based systems [11], [21].

Figure 1
Macroscopic Visual Observation of the Synthesized Starch and *Aloe Vera* Hydrogel After Incubation at 37 °C

Statistical Analysis

Statistical analysis was performed to evaluate differences among hydrogel formulations. One-way analysis of variance (ANOVA) was applied to the experimental data, and differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The characterization results for the hydrogel formulations reveal how composition affects their physicochemical, electrical, and antibacterial properties. The results of the experiment are examined in connection with changes in starch composition, citric acid concentration, and *Aloe vera* content. The evaluation of the hydrogel system for possible biomedical applications is enabled by each analysis, which demonstrates how formulation parameters affect the observed material properties.

FT-IR Analysis

The FT-IR spectra of the hydrogel formulations exhibited characteristic absorption bands of the starch-based matrix. A characteristic absorption band observed near 1638 cm^{-1} may be associated with carbonyl-related vibrations linked to interactions between starch and citric acid within the polymeric network [11], [15], [17]. This observation suggests the formation of partially crosslinked polymeric networks within the hydrogel system. Representative FT-IR spectra of selected hydrogel formulations are shown in Figure 2.

Furthermore, a broad band in the 3200–3500 cm^{-1} region was associated with O–H stretching vibrations and hydrogen-bonding interactions, contributing to the material's hydrophilic nature [12], [17]. The persistence of this band after curing suggests that not all hydroxyl groups participated in the esterification reaction, which is consistent with a partially modified polymeric network.

Minor differences in peak intensity among formulations were observed and may be associated with variations in composition, particularly in citric acid and *Aloe vera* content. However, no significant peak shifts were detected, suggesting chemical compatibility among the components and preservation of the primary polysaccharide structure [12], [15].

Swelling Analysis

The swelling behavior of the hydrogel formulations was evaluated after 24 hours of immersion in water. Major differences in structural stability were observed among the formulations. While some hydrogels retained partial integrity after immersion, others dissolved, surface-eroded, or experienced substantial mass loss, indicating that polymer degradation dominated stable swelling under the tested conditions.

The swelling response was strongly influenced by formulation composition. Formulations containing higher citric acid concentrations generally exhibited lower swelling and improved structural retention, suggesting that increased crosslinking density restricted polymer chain mobility and reduced water diffusion within the matrix [11], [15]. In contrast, formulations with lower effective crosslinking density absorbed more water but were more susceptible to dissolution and matrix disruption, indicating insufficient network stabilization.

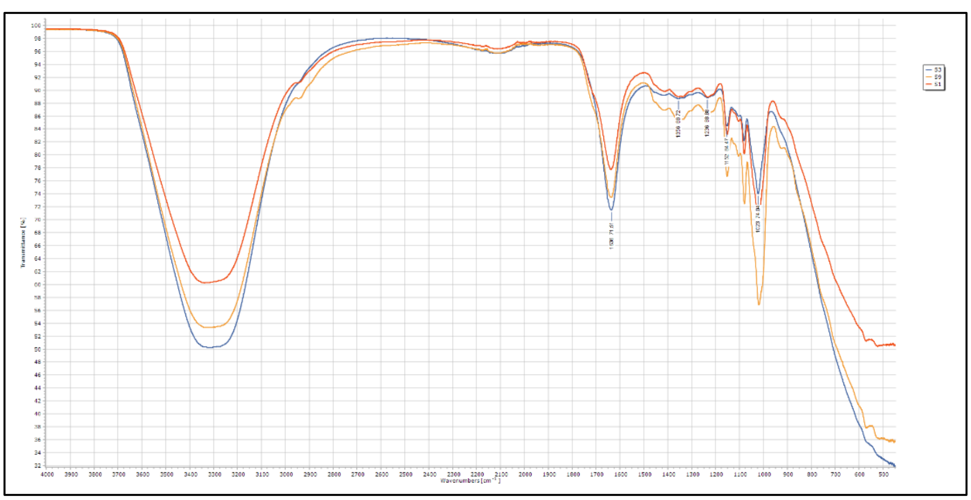

Figure 2
FT-IR Spectra of Hydrogel Formulations S1, S3, And S9 Showing Characteristic Absorption Bands of the Starch-Based Hydrogel System

Aloe vera incorporation also influenced swelling behavior due to the hydrophilic nature of its bioactive constituents [14]. In some formulations, increased hydrophilicity appeared to contribute to structural weakening and higher mass loss during immersion.

Overall, the results indicate that the swelling response depended on the balance between water absorption, crosslinking density, and matrix stability. Further optimization of formulation composition and curing conditions is required to improve aqueous stability and reduce polymer dissolution [5], [12].

pH Measurement

The surface pH of the hydrogel formulations ranged from 2.02 to 3.55, indicating a consistently acidic environment across all samples. This behavior is primarily attributed to residual citric acid within the polymeric matrix, which contributes to the system's overall acidity [11], [15].

Statistical analysis using one-way ANOVA revealed a significant difference among the formulations ($F = 5.09$, $p = 0.0011$), confirming that variations in composition influenced the chemical environment of the hydrogels. Differences in pH may be associated with changes in citric acid concentration and *Aloe vera* incorporation, which can affect the distribution of functional groups within the polymeric network [17], [14].

Most formulations exhibited relatively low variability, indicating acceptable reproducibility among measurements. However, several samples showed higher standard deviation values, suggesting possible heterogeneity within the hydrogel matrix.

Although the acidic character of the formulations may influence intermolecular interactions and crosslinking behavior, the observed pH range remains considerably lower than the physiological pH typically required for direct tissue-contact applications [14]. Therefore, the current system may require additional post-processing steps, such as neutralization, washing after thermal curing, or reduction of residual citric acid content, before considering potential biomedical or tissue-related applications [14]. Further optimization would be necessary to improve biocompatibility and suitability for biomaterials engineering applications.

Electrical Characterization

The electrical properties of the hydrogel formulations were analyzed to evaluate their electrical response and ability to support ionic charge transport. The results revealed a strong dependence of the electrical behavior on both frequency and composition, which is characteristic of hydrated polymer systems [4], [10]. The observed response reflects the mobility of ionic species within the hydrogel network, influenced by the presence of water and ionic components associated with citric acid and *Aloe vera* [10], [13].

Variations among formulations indicate that compositional changes affected the material's electrical behavior. In particular, the incorporation of *Aloe vera* appeared to favor ionic transport, while variations in citric acid concentration may have influenced the internal organization and density of the polymer network [10], [12]. These findings demonstrate that the electrical response of the hydrogel system can be modulated through formulation variation, which may be relevant for applications involving electrically responsive biomaterials.

Impedance

The impedance response of the hydrogel formulations exhibited a clear frequency dependence, with higher impedance values observed at low frequencies (20 Hz). This behavior is characteristic of hydrated polymeric systems, where interfacial polarization and restricted ionic mobility limit charge transport [4], [10]. As frequency increased, impedance progressively decreased, indicating improved ionic conduction through the hydrogel matrix.

Differences among formulations indicate that composition significantly influenced the electrical response of the materials. Formulations S3 and S9 exhibited comparatively lower impedance values, suggesting more efficient ionic transport within the polymeric network. This behavior may be associated with a more favorable balance between water retention, *Aloe vera* incorporation, and crosslinking density, which together can facilitate ion mobility [10], [13].

In contrast, formulations S5 and S8 showed higher impedance values, indicating a more resistive internal structure. This response may be related to increased network compactness or reduced mobility of ionic species within the matrix, limiting conductive pathways and charge displacement [12], [15]. Variations in citric acid concentration may also have influenced the organization of the polymer network and ion diffusion throughout the material. Overall, the impedance results demonstrate that relatively small compositional changes can significantly affect ionic transport behavior and internal network organization within starch-based hydrogel systems (Table 2).

Table 2
Measured Impedance Values

Formulation	Impedance (kΩ)
S1	7.69
S2	5.88
S3	4.55
S4	9.09
S5	12.50
S6	11.11
S7	7.14
S8	16.67
S9	5.26
S10	10.00

Conductance Analysis

The conductance of the hydrogel formulations was determined from impedance measurements and varied with composition (Table 3). Formulations S3 and S9 exhibited comparatively higher conductance values, suggesting more effective ionic transport within the hydrated polymer network [4], [10]. This behavior may be associated with a more balanced interaction between water retention and crosslinking density, which could facilitate ion mobility throughout the matrix. In addition, the incorporation of *Aloe vera* may have contributed to the presence of additional hydrophilic and ionic constituents that enhance charge transport.

Table 3
Measured Conductance Values

Formulation	Conductance (mS)
S1	0.13
S2	0.17
S3	0.22
S4	0.11
S5	0.08
S6	0.09
S7	0.14
S8	0.06
S9	0.19
S10	0.10

In contrast, formulations with lower conductance values likely presented a more compact or less hydrated internal structure, restricting conductive pathways and reducing ionic mobility within the matrix [10], [20]. Variations in citric acid concentration may also have affected the degree of crosslinking, thereby influencing the organization of the polymeric network and limiting ion diffusion. Overall, the results indicate that relatively small compositional changes can significantly alter charge-transport behavior in starch-based hydrogel systems.

Antibacterial Analysis

The antibacterial activity of the hydrogel formulations was qualitatively evaluated against *Escherichia coli* using the disk diffusion method. The untreated control exhibited a dense, uniform bacterial lawn, whereas the hydrogel-treated samples showed visible regions of reduced bacterial growth surrounding the materials (Figure 3).

The observed response may be associated with the combined presence of *Aloe vera* and the acidic environment generated by residual citric acid within the hydrogel matrix. Formulations containing *Aloe vera* showed visible differences in bacterial growth compared with the matrix control, suggesting that incorporating bioactive compounds may influence bacterial proliferation [10], [14].

However, no quantitative measurements of inhibition zones were performed during this study. Therefore, the results suggest a qualitative reduction in bacterial growth; however, quantitative inhibition zone measurements are required to confirm antibacterial activity and establish direct comparisons among formulations. Additional microbiological analysis would also be necessary to evaluate the reproducibility and extent of the observed bacterial response.

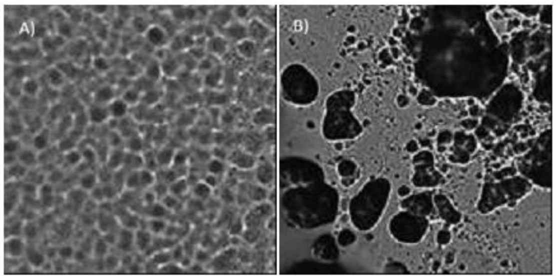

Figure 3
Microscopic Image of: A) *Escherichia Coli* Growth (Positive Control), Showing a Dense and Uniform Bacterial Distribution.; B) A Hydrogel-Treated Sample Showing Localized Regions of Reduced or Absent Bacterial Growth

Microscopic observations supported these findings, showing localized areas of reduced bacterial presence at the hydrogel–bacteria interface. These results are consistent with the macroscopic observations, which indicate a qualitative antibacterial response. Further quantitative and statistical analyses are required to confirm the extent of antimicrobial activity and its dependence on formulation composition.

CONCLUSIONS

This study developed bio-based hydrogels composed of cassava starch, citric acid, and *Aloe vera* through a thermally induced synthesis process. The results demonstrated that formulation composition influenced the physicochemical, electrical, and qualitative antibacterial behavior of the hydrogel systems. FT-IR analysis suggested the formation of ester linkages within the polymeric network, while swelling and pH measurements revealed formulation-dependent differences in aqueous stability and chemical environment.

Electrical characterization showed frequency-dependent impedance and conductance responses associated with variations in crosslinking density and *Aloe vera* incorporation. Qualitative antibacterial evaluation against *Escherichia coli* showed visible reductions in bacterial growth surrounding several hydrogel formulations; however, quantitative inhibition measurements were not performed.

These findings suggest that the proposed starch–citric acid–*Aloe vera* system may serve as a preliminary platform for bio-based hydrogel development; however, further optimization, neutralization of residual acidity, mechanical testing, quantitative antimicrobial analysis, controlled degradation studies, cytotoxicity evaluation, and biocompatibility testing are required before considering tissue repair applications.

LIMITATIONS AND FUTURE WORK

This study has several limitations related to the experimental conditions and the scope of characterization. The hydrogels were prepared and cured using a conventional laboratory oven, which may have contributed to non-uniform heat distribution and affected the consistency of the hydrogel networks. Additionally, the hydrogels were fabricated using standard glass beakers rather than standardized molds or Petri dishes, which may have influenced sample geometry, thickness, and reproducibility during swelling and antibacterial evaluations.

The antibacterial evaluation was limited to *E. coli* and was performed qualitatively. Therefore, quantitative measurements such as inhibition zone diameters were not obtained. In addition, electrical characterization was limited to impedance and conductance measurements at selected frequencies. Further frequency-dependent analysis and equivalent circuit modeling could provide a more detailed understanding of the hydrogel's electrical behavior.

Future studies should focus on improving process control by using more precise heating equipment and standardized fabrication methods to produce more uniform samples. Antibacterial evaluation should also be expanded to include additional microorganisms together with quantitative analysis. Furthermore, additional studies involving mechanical characterization, controlled release of *Aloe vera* bioactives, controlled degradation, cytotoxicity, and biocompatibility are necessary before considering advanced biomedical applications.

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