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The potential of smart scaffolds containing quince seed mucilage and kappa-carrageenan for cell culture under normal physiologic conditions and varying pH levels

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ABSTRACT

The purpose of this study is to investigate the ability of smart scaffolds of Kappa-carrageenan (Carr) and the combination of Kappa-carrageenan and quince seed mucilage (Carr:Quc) to support C2C12 viability and growth for cultured meat production. Carr and Carr:Quc with a final concentration of 1.5% (*v/w*) were developed using a 5% potassium chloride solution. The capability of the scaffolds to respond to the pH change of the environment was evaluated, and the viability of C2C12 at normal pH (7.4) and varying pH levels (7.4-5.5) was assessed. The evaluation of swelling changes with varying pH (pH 1-7) showed that for the Carr scaffold, the highest swelling was observed at pH 5, reaching 145%, which showed a significant difference compared to swelling at other pH levels ($p < 0.05$). The highest swelling for the Carr:Quc scaffold was also observed at pH 5, reaching 428%, with a significant difference compared to swelling at other pH levels ($p < 0.05$). Moreover, the change in the swelling behavior of the scaffolds was evaluated by changing the pH from 7.4 to 5.5. Carr did not show any swelling change, while Carr:Quc demonstrated a significant change in swelling after exposure to pH 5.5 for 30, 45, 60, 180, and 360 min. On Carr:Quc, C2C12 showed higher viability in normal conditions compared to varying pH levels from 7.4 to 5.5. Furthermore, after culturing on Carr:Quc, C2C12 maintained their viability throughout the culture period for 15 days at pH 7.4 and showed the potential for spheroid formation. The findings of this study could pave the way for the design of scaffolds made of edible biopolymers to facilitate tissue engineering of cultured meat.

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1-Introduction

In today's rapidly evolving world, one of the most significant challenges is the urgent need for sustainable protein sources that reduce the environmental impact of traditional meat production. With the global population increasing and environmental concerns escalating, conventional methods reliant on animal slaughter are contributing to environmental degradation at an unsustainable rate. An innovative solution is cultivated meat, which aligns with principles of sustainability, health, and ethics (1, 2). This approach requires advanced technologies, such as smart scaffolds, which enhance tissue quality and adapt dynamically to environmental changes.

Smart scaffolds play a critical role in supporting cell growth, proliferation, and differentiation, optimizing outcomes in tissue engineering (3, 4). Since stem cells, myoblasts, and muscle progenitor cells are anchorage-dependent, substrates or scaffolds must facilitate their attachment, proliferation, and differentiation. These substrates must possess mechanical strength, porosity, and biocompatibility to guide cellular behavior effectively. Among these, edible, porous, and environmentally responsive scaffolds have gained attention due to their ability to exhibit at least a 10% change in surface area in response to pH or temperature variations. This adaptability enhances adhesion, proliferation, and differentiation.

Although the precise mechanisms remain unclear, it is hypothesized that scaffold surface changes and mechanical stimuli enhance cell proliferation and differentiation (3). For instance, periodic mechanical tension applied to hydrogel-based scaffolds influences cell proliferation and modulates protein expression, such as elastin and collagen. Additionally, extracellular kinase phosphorylation can accelerate cell proliferation independently (5, 6, 7). Smart biopolymers have garnered attention in tissue engineering and regenerative medicine due to their dynamic responsiveness to environmental conditions. This enables them to interact effectively with biological systems, mimicking the extracellular matrix (ECM) to provide a supportive microenvironment for cell adhesion, growth, and proliferation (8).

In cultivated meat production, smart biopolymers address challenges like preventing cellular atrophy during culture. Maintaining cell health, preventing atrophy, and promoting robust growth are primary obstacles in efficient cell culture systems. Environmentally responsive biopolymers optimize these processes by inducing controlled contractions in the culture environment, supporting healthy myofiber formation and mitigating atrophy. These mechanical stimuli enhance muscle cell growth and differentiation, improving the quality and efficiency of cultivated meat production (3).

Among responsive biopolymers, pH-sensitive polymers demonstrate efficacy in enhancing scaffolds and promoting muscle cell growth. These biopolymers contain ionizable groups, enabling them to act as hydrogen donors or acceptors depending on environmental conditions. For example, anionic polymers with carboxylic (–COOH) or sulfate ($–SO_3^-$) groups accept hydrogen ions at low pH levels and release them at higher pH levels. Consequently, they swell more as pH increases, making them adaptable for dynamic applications (9, 10). As pH rises, carboxylic acid group ionization induces electrostatic repulsion between polymer chains, disrupting hydrogen bonds and causing expansion. This facilitates water infiltration (11).

Smart natural polysaccharides, particularly marine-derived ones, have attracted attention due to their low toxicity and excellent biocompatibility. These polysaccharides are widely used in food, pharmaceuticals, and biomedicine. Carrageenan, extracted from red algae, is renowned for its unique properties. Kappa-carrageenan, a sulfated galactan, forms thermally reversible gels upon cooling due to a coil-to-helix transition. This transformation, regulated by temperature and ionic content, results in stable gel formation (11, 12, 13).

Similarly, plant seed-derived polysaccharides, such as quince seed mucilage, have gained attention in tissue engineering and cell encapsulation due to their high biocompatibility (14, 15). Quince seed mucilage is a promising biomaterial due to its biodegradability, non-toxicity, and bioavailability. Its significant uronic acid content classifies it as a smart biopolymer sensitive to pH changes. Studies show minimal swelling at pH 1.2 and maximum swelling at pH 7.4 (16, 17). Combining kappa-carrageenan and quince seed mucilage forms a hydrogel containing sulfate and carboxylic acid groups, making it responsive to pH variations.

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Research shows that scaffolds made of materials like alginate, chitosan, cellulose, or collagen, which exhibit at least a 10% surface area change in response to slight pH or temperature variations, induce mechanical stress in the culture environment. Periodic application of this stress enhances cell adhesion and proliferation, improving tissue engineering outcomes (3).

This study evaluates the effect of pH changes on the swelling behavior (mechanical tension) of smart kappa-carrageenan (Carr) scaffolds and composite scaffolds made of quince seed mucilage and kappa-carrageenan (Carr:Quc). Mouse myoblasts (C2C12) cultured on these scaffolds will be assessed under normal conditions (pH 7.4) and within a physiological pH range (5.5–7.4) to determine their impact on cell proliferation. Additionally, cell viability and proliferation on the Carr:Quc scaffold will be monitored over 15 days to evaluate its potential as a novel scaffold for cultivated meat production.

2- Materials and Methods

2.1. Materials

Quince seeds were purchased from a herbal pharmacy in Isfahan. Kappa-carrageenan powder, branded BLG, from South Korea, was utilized. Potassium chloride, sodium phosphate buffer, and hydrochloric acid were obtained from Merck, Germany. The C2C12 cell line was acquired from ATCC, USA. Materials required for cell culture, including Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), trypsin enzyme, and penicillin-streptomycin antibiotics, were sourced from Gibco, USA. For cell staining, materials such as Calcein-AM, ethidium bromide, paraformaldehyde, Triton X-100, DAPI, phalloidin, and Hoechst were procured from Thermo Fisher Scientific, USA.

2.2. Extraction of quince seed mucilage

Quince seed mucilage was extracted using deionized warm water. The seeds were immersed in warm water at 50°C for 30 min and continuously stirred to facilitate the release of mucilage. Subsequently, the mucilage was separated from the seeds using a laboratory extractor. The extracted mucilage was freeze-dried (Dena Vacuum, Iran) and then ground into a fine powder (18).

2.3. Preparation of kappa-carrageenan hydrogel (Carr) and composite hydrogel of kappa-carrageenan and quince seed mucilage (Carr:Quc)

To prepare the kappa-carrageenan hydrogel (Carr), a specific amount of kappa-carrageenan powder was dissolved in deionized water to achieve a final concentration of 1.5% (w/v). For the composite hydrogel (Carr:Quc), kappa-carrageenan powder and quince seed mucilage were mixed in a 1:1 ratio, resulting in a final concentration of 1.5% (w/v). The mixture was dissolved in deionized water to form a homogeneous colloidal solution. Both solutions were stirred at 60°C for 30 min to ensure uniform particle distribution. The hot Carr and Carr:Quc solutions were poured separately into circular molds made of polymethyl methacrylate, each with a diameter of 17 mm. The solutions were allowed to cool at room temperature for 15 min. To form hydrogels, the samples were immersed in a 5% (w/v) potassium chloride (KCl) bath for one hour at room temperature. The resulting hydrogels were dried in a vacuum oven at 50°C and subsequently used for further evaluations (19).

2.4. Evaluation of scaffold swelling behavior with pH changes

The swelling behavior of the scaffolds was assessed at pH levels of 1, 2, 3, 4, 5, 6, and 7. After preparation, the hydrogels were dried under vacuum and then immersed in buffer solutions with the desired pH values for 24 h at 37°C. Following immersion, the hydrogels were weighed (W1), dried again in an oven at 50 °C for 24 h, and reweighed (W2). The swelling percentage of the hydrogels was calculated using Equation 1 (13):

$$\text{Swelling (\%)} = [(W1 - W2) / W2] \times 100 \quad (1)$$

2.5. Assessment of scaffold swelling variations with changes in pH from 7.4 to 5.5

To evaluate the swelling variations of the scaffolds in response to pH changes from 7.4 to 5.5, Carr and Carr:Quc hydrogels were prepared and dried under vacuum. Eight different groups of both hydrogels were placed in sodium phosphate buffer at pH 7.4 and 37 °C for 24 h. After this initial incubation period, the hydrogels were removed, weighed, and immediately transferred to sodium phosphate buffer at pH 5.5 and 37 °C for varying durations, including 5, 15, 30, 45, 60, 180, 360, and 480 min. At each specified time interval, the hydrogels were removed, weighed again, and analyzed to determine which groups

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exhibited an increase in swelling. Table 1 summarizes the experimental conditions for the eight treatment groups applied to both hydrogels.

Table 1- pH change conditions for different treatments

treatment	Conditions
TRT1	24 h in pH 7.4 and 5 min in pH 5.5
TRT2	24 h in pH 7.4 and 15 min in pH 5.5
TRT3	24 h in pH 7.4 and 30 min in pH 5.5
TRT4	24 h in pH 7.4 and 45 min in pH 5.5
TRT5	24 h in pH 7.4 and 60 min in pH 5.5
TRT6	24 h in pH 7.4 and 180 min in pH 5.5
TRT7	24 h in pH 7.4 and 360 min in pH 5.5

2.6. Cell viability assessment on the scaffolds under pH change conditions

To evaluate cell viability under varying pH conditions, 1,000,000 cells were seeded onto the Carr:Quc hydrogel and incubated under proliferative conditions at pH 7.4 for 24 h. Subsequently, the culture medium was replaced with a growth medium adjusted to pH 5.5 using 1N HCl. This pH 5.5 condition was maintained for 15 min. Afterward, the medium was removed, and the scaffolds were gently washed with phosphate-buffered saline (PBS). Regular growth medium at pH 7.4 was then reintroduced, and the scaffolds were incubated under normal proliferative conditions for an additional 24 hours. As a control, Carr:Quc hydrogels were incubated under standard proliferative conditions (pH 7.4) for 48 h. Finally, cell viability was assessed using Calcein-AM and Ethidium Bromide staining, and fluorescence imaging was performed using a fluorescence microscope (Echo Revolution, USA) (20).

2.7. Cell viability assessment on Carr:Quc under normal conditions

To evaluate cell adhesion and morphology, cell nuclei and filamentous actin proteins were stained using DAPI and Phalloidin, respectively. Specifically, 1,000,000 cells were seeded onto the scaffolds. One day post-seeding, the cells were fixed with 4% paraformaldehyde for 15 min. The scaffolds were then washed with PBS, and 0.1% Triton X-100 solution was applied to permeabilize the cell membranes. Following another PBS wash, Phalloidin stain was added and incubated for one hour. The scaffolds were subsequently washed with PBS, and DAPI stain was applied for 10 min before a final PBS wash. Imaging was performed using a confocal microscope (ZEISS, USA).

Additionally, to assess cell viability over a 15-day period, Calcein-AM and Ethidium Bromide staining were employed, followed by imaging using a fluorescence microscope. Calcein-AM and Hoechst staining were also conducted on days 1 and 14 of cell culture. For this purpose, a solution of 1 mM Calcein-AM and 20 mM Hoechst was diluted at a 1:1000 ratio in complete culture medium and added to the cell growth medium. The cells were incubated for 15 min at 37 °C with 5 % CO_2 and subsequently imaged using a confocal microscope. To analyze cell morphology, a scanning electron microscope (SEM; ZEISS, Germany) was utilized. On day 14 of cell culture, the scaffolds were removed from the culture medium, washed with PBS, and dried using a freeze-dryer prior to imaging. The samples were then imaged at a magnification of 10 µm (21).

2.8. Statistical analysis

Statistical analysis was performed using SAS software. Depending on the experimental design, either a Student's t-test or one-way analysis of variance (ANOVA) with the least significant difference (LSD) test was conducted. Graphs were generated using GraphPad Prism software and presented as bar charts with standard deviation (s.d.) based on at least three replicates. Statistical significance was defined as $p < 0.05$.

3-Results and discussion

3.1. Evaluation of the effect of pH changes on scaffold swelling

Figure 1-a illustrates the swelling behavior of the Carr hydrogel in response to pH changes. The hydrogels were incubated at various pH levels for 24 hours at 37 °C. As observed, no swelling occurred at pH 1 and 2, indicating that the hydrogel was unable to absorb water under these conditions. However, as the pH increased, a progressive rise in water absorption was

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noted. The swelling percentage reached 108% at pH 3, 126% at pH 4, and approximately 145% at pH 5. Beyond this point, as the pH continued to increase, a decline in swelling was observed, with the swelling percentage decreasing to approximately 90% at pH 7.

Similarly, Figure 1-b depicts the swelling behavior of the Carr:Quc hydrogel in response to pH changes. A comparable trend was observed for this hydrogel, albeit with some notable differences. Specifically, the Carr:Quc hydrogel exhibited a swelling percentage of approximately 35% at pH 1 and around 125% at pH 2. Swelling increased progressively with rising pH, reaching a maximum of approximately 428% at pH 5. However, as the pH continued to rise beyond this point, the swelling percentage decreased, reaching approximately 325% at pH 7.

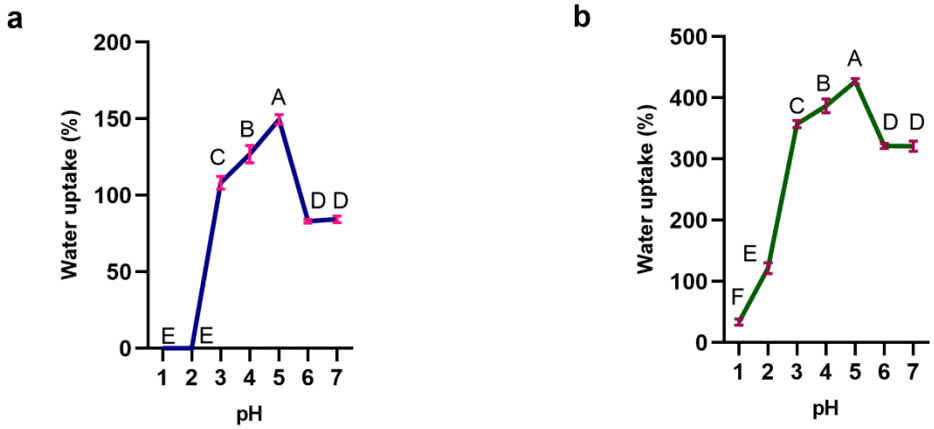

Figure 1. Swelling change of the Carr scaffold (a) and the Carr:Quc scaffold (b) with pH changes. Different letters indicate significant differences between treatments ($p < 0.05$). Statistical significance was determined using one-way ANOVA.

The trend in swelling or water uptake percentages observed for the Carr and Carr:Quc scaffolds aligns with mechanisms reported in various studies. Both hydrogels contain anionic polymers, which include acidic functional groups such as carboxylic acid (–COOH) and sulfate (–OSO_3H). In acidic pH conditions, these groups retain hydrogen within their structure, existing as COOH and OSO_3H. Conversely, at higher pH levels, they begin to lose protons, converting to COO^- and OSO_3^-. As the ionization of carboxylic acid and sulfate groups increases, electrostatic repulsion between polymer chains also intensifies, leading to the disruption of hydrogen bonds. Consequently, the polymer structure expands, facilitating greater water penetration (12).

The water uptake behavior observed across the pH range of 1–7 demonstrated that both the Carr and Carr:Quc scaffolds exhibited higher water absorption at pH 5 compared to pH 7. In pH-sensitive biopolymers, the ionization of acidic or basic functional groups within the hydrogel structure determines their water absorption capacity. For the Carr and Carr:Quc hydrogels, it is likely that the ionization of acidic functional groups—and consequently the electrostatic repulsion—was more pronounced at pH 5 than at pH 7. This increased ionization facilitated hydrophilic interactions between COO^- and SO_3^- groups and water molecules, resulting in significant water absorption.

Numerous studies have highlighted the utility of smart, pH-sensitive biopolymers, particularly in drug delivery systems. For instance, Lohani et al. (2016) developed a pH-sensitive hydrogel network using kappa-carrageenan and sodium carboxymethyl cellulose. They investigated the release of ibuprofen from the hydrogel at pH 7.4 and 1.2. Their findings indicated that the hydrogel swelled by approximately 700% at pH 7.4 but only about 200% at pH 1.2. At pH 7.4, the ionization of carboxylic acid groups led to enhanced water penetration, network expansion, and complete drug release, whereas at pH 1.2, the release was limited due to reduced swelling (22). Similarly, another study utilized a hydrogel network composed of kappa-carrageenan and carboxymethyl cellulose for probiotic drug delivery. The aim was to deliver the probiotic bacterium *Lactobacillus plantarum* to the colon. The swelling and drug release behavior were examined at pH 7.4 and 1.2. Results showed significantly higher swelling and drug release at pH 7.4, reaching

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600 CFU/g, compared to 180 CFU/g at pH 1.2, where the network remained compact and restricted drug release (12).

3.2. Evaluation of swelling changes of scaffolds due to pH change from 7.4 to 5.5

Based on previous results, pH levels of 7.4 and 5.5 were selected as critical points for evaluating pH-induced swelling changes. The pH range of 7.4 to 5.5 is considered physiological, with pH 7.4 representing normal physiological conditions and optimal cell culture environments. In contrast, pH 5.5 corresponds to abnormal physiological conditions, such as inflammatory responses or wound healing, where the pH of the affected area may decrease to this level. Given the prior findings indicating maximum swelling at pH 5 and the relevance of the physiological pH range, pH 7.4 and 5.5 were chosen for further investigation of pH-induced swelling variations.

Figure 2 illustrates the swelling changes of the Carr scaffold when transitioning from pH 7.4 to 5.5 over various time intervals. All treatments were initially incubated at pH 7.4 and 37 °C for 24 h, followed by exposure to pH 5.5 and 37 °C for durations of 5, 15, 30, 45, 60, 180, or 360 min, after which swelling was measured. As shown, no significant increase in swelling was observed for any of the treatments. Similarly, Figure 3 demonstrates the swelling changes for the Carr:Quc scaffold under the same conditions. Unlike the Carr scaffold, significant swelling was observed for all treatments except for the 5-min interval at pH 5.5 (TRT1). Based on these findings, only the Carr:Quc scaffold was selected for further evaluation of cell viability under pH changes from 7.4 to 5.5, as no significant differences in swelling were observed for the Carr scaffold under these conditions.

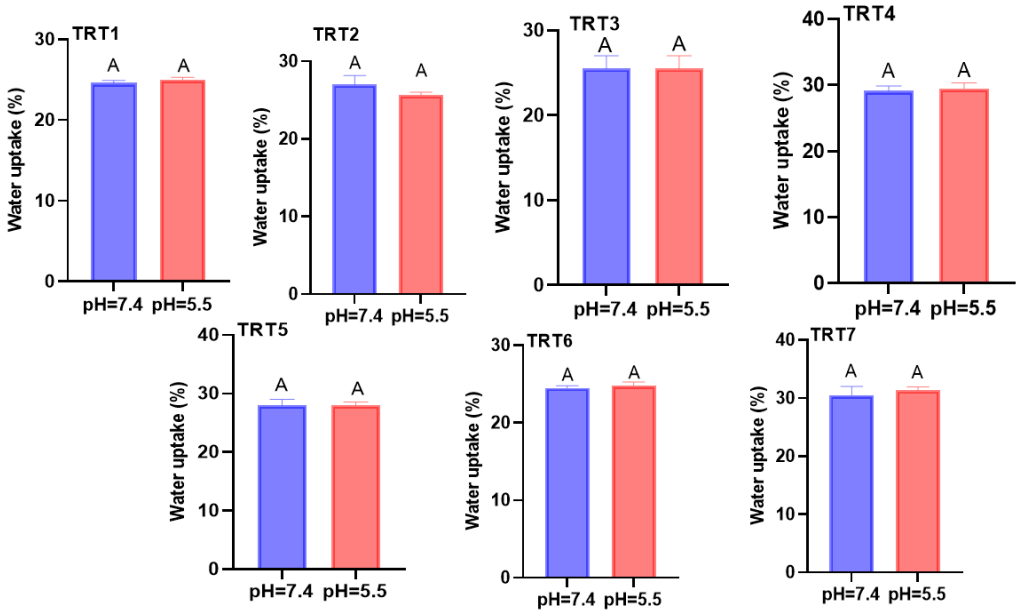

Figure 2. Swelling change of the Carr scaffold from pH 7.4 to pH 5.5. The same letters indicate no significant difference between treatments ($p > 0.05$). Statistical significance was determined using the *t*-Student test.

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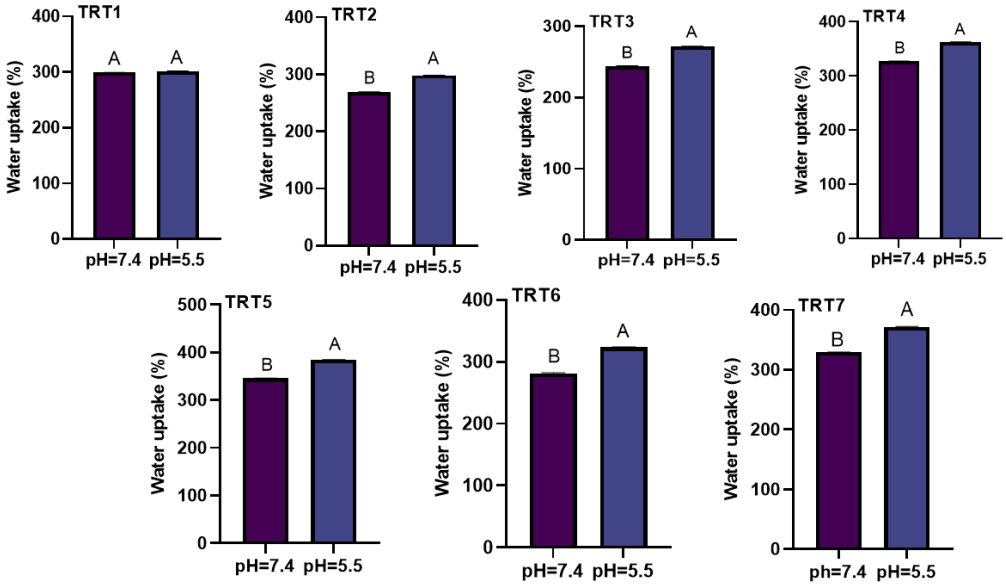

Figure 3. Swelling variation of the Carr:Quc scaffold from pH 7.4 to pH 5.5. Different letters indicate significant differences between treatments ($p < 0.05$). Statistical significance was determined using the *t*-Student test.

3.3. Evaluation of cell viability on the Carr:Quc scaffold under normal conditions and pH change from 7.4 to 5.5

Figures 4-a and 4-b illustrate live cells (green) and dead cells (red) 48 h after seeding under two distinct conditions: normal physiological conditions at pH 7.4 (Figure 4-a) and during pH variation from 7.4 to 5.5 (Figure 4-b). As shown in Figure 4-a, under normal conditions, the cells exhibited robust proliferation, with only a small proportion of cells undergoing cell death. However, when the pH of the culture medium was altered to acidic conditions at pH 5.5 for 15 min (Figure 4-b), a significantly higher proportion of cells died, resulting in a marked decrease in cell viability. These findings highlight the detrimental impact of acidic pH conditions on cell survival and underscore the importance of maintaining optimal pH levels for cell culture applications.

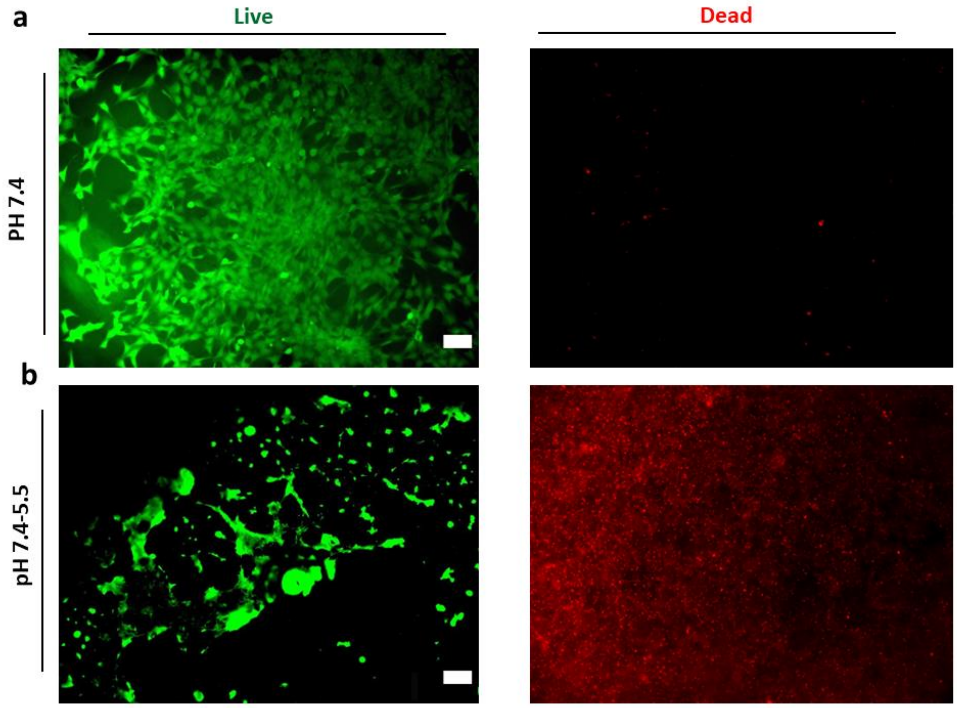

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Figure 4. Fluorescence images of live cells (green) and dead cells (red) on the Carr:Quc scaffold: (a) at pH 7.4 and (b) after pH change from 7.4 to 5.5. Scale bar: 100 µm.

Numerous studies have demonstrated that environmental pH changes in pH-sensitive scaffolds can enhance cell proliferation, orientation, and differentiation. For instance, in one study, a synthetic pH-sensitive hydrogel composed of dimethylaminoethyl methacrylate (DMAEMA)/2-hydroxyethyl methacrylate in ratios of 30/70, 20/80, and 10/90 was developed, and the viability and proliferation of NIH/3T3 mouse fibroblasts were evaluated under acidic conditions. The results revealed that when the pH of the cell growth medium decreased to approximately 5.5 or 6 over three days, cell proliferation was higher than under normal pH conditions. This effect was attributed to the response of the smart scaffolds to pH changes, which induced swelling and enhanced oxygen and nutrient transport to the scaffold's core, thereby promoting cell proliferation. Notably, the 30/70 ratio exhibited the most pronounced response to pH changes (23).

However, in the present study, the Carr:Quc hydrogel did not demonstrate a similar effect. On the contrary, altering the optimal cell growth conditions resulted in significant cell death. When cells die due to pH changes in the culture environment, it suggests that the pH-responsive scaffolds either do not function optimally or that the pH range exceeds the tolerance threshold of the cells. Several factors may contribute to this outcome. For example, extreme pH changes can induce cellular stress, leading to apoptosis or necrosis. Most cells thrive within a narrow pH range of 7.2 to 7.4, and deviations from this range can disrupt cellular homeostasis, resulting in stress-induced cell death. Furthermore, pH fluctuations can create an unstable microenvironment, whereas cells require a stable environment for growth and proliferation. Rapid swelling or contraction of scaffolds can also impose osmotic stress, potentially damaging the cell membrane. Additionally, if scaffolds fail to facilitate adequate diffusion of nutrients and oxygen, central cells may become deprived of essential resources, leading to cell death.

Given these observations, while the Carr:Quc scaffold responded effectively to pH changes and exhibited significant swelling, the cultured cells were unable to tolerate pH conditions outside the normal physiological range, ultimately leading to apoptosis. Nevertheless, considering the results and the fact that the scaffolds maintain a closed network at pH 1 and exhibit substantial swelling and opening at pH 7, these scaffolds hold potential applications as microcarriers in oral drug delivery systems.

3.4. Evaluation of cell viability on the Carr:Quc scaffold under normal conditions

Figure 5 illustrates cell adhesion and distribution within the Carr:Quc hydrogel one day after cell seeding. Figure 5-a demonstrates proper adhesion of cells to the scaffold surface, along with the formation of filamentous actin. Figure 5-b highlights the different layers of the scaffold, including cells located in the inner sections. This figure represents various states of nuclei and filamentous actin across layers along the Z-axis. Filamentous actin, one of the most critical components of the cytoskeleton, plays a key role in essential cellular processes such as migration, adhesion, and signaling (24).

The composition and properties of hydrogels, including their mechanical characteristics, stiffness, and biocompatibility, significantly influence the formation of filamentous actin. Cells exhibit varying responses to different hydrogels, and appropriately designed hydrogels can enhance cytoskeletal reorganization (1). Additionally, the degradation rate of hydrogels is another crucial factor. If a hydrogel degrades too rapidly, it may fail to provide a stable structure for organizing filamentous actin. Conversely, a hydrogel with a degradation rate aligned with the cellular regeneration capacity can facilitate proper cytoskeletal formation. Furthermore, the three-dimensional matrix structure of hydrogels impacts how cells interact with their surrounding environment. A hydrogel with an optimal structure can promote cell migration and the formation of actin-rich structures. These findings suggest that the Carr:Quc hydrogel provides a favorable microenvironment for supporting cytoskeletal organization in cells.

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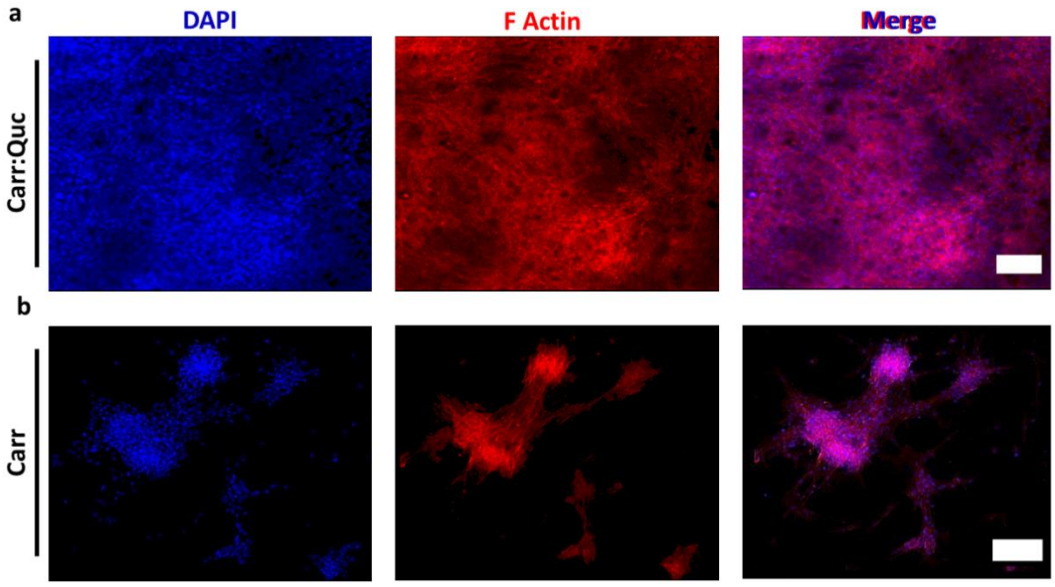

Figure 5. (a) Confocal microscopy images of cells 1 day after seeding on the Carr:Quc scaffold. (b) Nuclei and filamentous actin in different layers of the scaffold. Scale bar: 100 µm.

Moreover, Figure 6-a demonstrates cell viability and growth over a 15-day period on the Carr:Quc scaffold. As shown, the cells maintained their viability throughout the culture period on the scaffold. By the second week of culture, the cells exhibited a tendency to form cell spheroids. On the first day, the cells displayed a stretched and flattened morphology, spreading across the scaffold surface. By day 14, the formation of cell spheroids was observed, and by day 15, distinct spheroid structures had developed, with no discernible single-cell morphology remaining. Figure 6-b provides a schematic representation of spheroid formation on natural hydrogel substrates. Integrin-extracellular matrix (ECM) interactions regulate cadherin expression, which plays a critical role in this process. Subsequently, cell aggregation and intercellular interactions lead to cadherin clustering. Finally, the ECM is remodeled, resulting in the emergence of a dense spheroid structure. In such dense spheroids, individual cell morphology is no longer distinguishable (25, 26).

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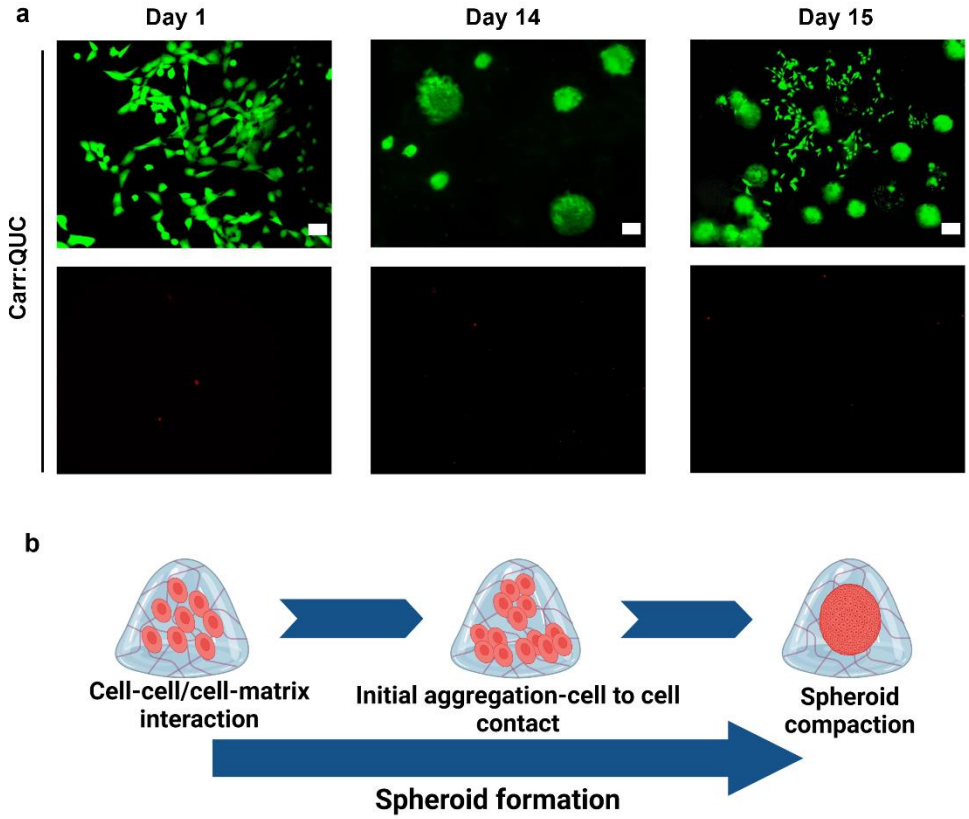

Figure 6. (a) Fluorescence images of live cells (green) and dead cells (red) on the Carr:Quc scaffold. Scale bars: 100 µm. (b) Spheroid formation procedures on a hydrogel structure.

Furthermore, Figure 7 shows confocal images of cells stained with Calcein-AM and Hoechst 1 and 14 days after seeding on the Carr:Quc hydrogel. On day 1, the cells exhibited a stretched and flattened morphology. However, by day 14, the cells had formed spheroids, confirming the progression of spheroid formation over time. Figure 8 also demonstrates an SEM image of a compact spheroid formed 14 days after cell seeding. As observed, individual cell morphology is not distinguishable within the spheroid structure.

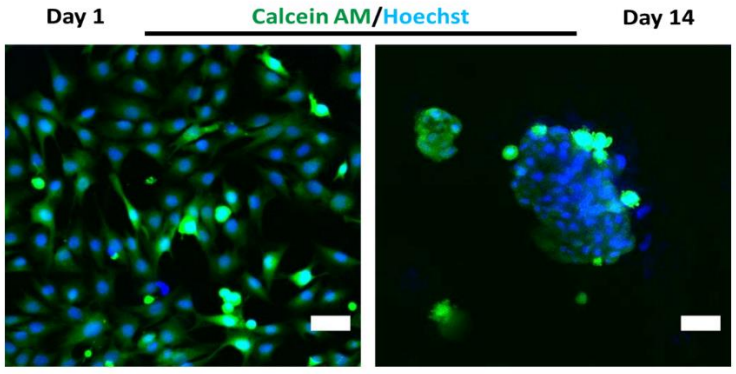

Figure 7. The cell morphology and spheroid formation process 1 and 14 days after seeding on the Carr:Quc scaffold. Scale bar: 100 µm.

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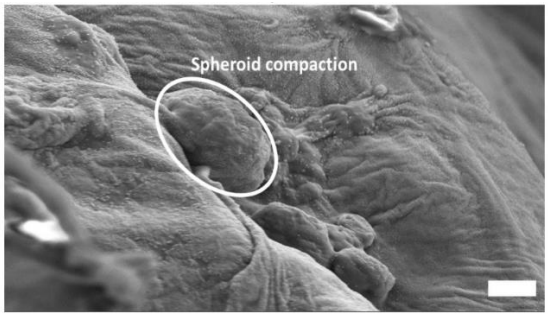

Figure 8. SEM image of a spheroid formed 14 days after seeding on the Carr:Quc scaffold. Scale bar: 100 µm.

These results demonstrated that cells were able to grow on the hydrogel over a two-week period, and the hydrogel exhibited no signs of toxicity. Furthermore, after two weeks in culture, the cells formed spheroids. Spheroid formation within hydrogel and natural polymer structures, such as collagen, hyaluronic acid, chitosan, and Matrigel, has also been widely reported (27, 28, 29). Several factors contribute to the process of spheroid formation on hydrogel substrates, including the following (30, 31):

1. Cell-cell interactions: The hydrogel matrix facilitates cell-cell interactions by creating conditions that bring cells into closer proximity. This promotes cell aggregation and the formation of multicellular structures.
2. Cell-matrix interactions: Cells interact with the hydrogel matrix through adhesive molecules present within the matrix. These interactions influence cellular behaviors such as migration and aggregation, ultimately contributing to spheroid formation.
3. Matrix structure: The physical properties of the hydrogel, including stiffness, porosity, and the type and size of pores, play a significant role. A biomimetic substrate with appropriate mechanical properties provides an optimal environment for cell aggregation and spheroid formation.
4. Oxygen and nutrient gradients: As the size of the spheroid increases, gradients of nutrients and oxygen form within the hydrogel matrix. This creates a microenvironment that more closely mimics physiological conditions, thereby influencing cell behavior and spheroid morphology.
5. Cell proliferation and programmed Cell Death (Apoptosis): The balance between cell proliferation and apoptosis also contributes to spheroid formation. Cells within the hydrogel matrix may proliferate at varying rates, and the equilibrium between these processes helps shape the spheroid structure.

4- Conclusion

The results demonstrated that the scaffold composed of kappa-carrageenan and quince seed mucilage holds significant potential for applications in cell culture and cellular agriculture. Since cells were able to proliferate effectively on this scaffold under normal conditions and during long-term culture, it could be considered a suitable candidate for the production of lab-grown meat.

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مقاله علمی-پژوهشی

امکان استفاده از داربست‌های هوشمند متشکل از موسیلاژ دانه به و کاپاکاراگینان به عنوان داربست سلولی در شرایط نرمال فیزیولوژیکی و تغییر pH

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چکیده

هدف از پژوهش حاضر بررسی قابلیت رشد سلولی بر روی داربست های هوشمند کاراگینان (Carr) و ترکیب کاراگینان و موسیلاژ دانه به (Carr:Quc) به منظور تولید گوشت آزمایشگاهی می‌باشد. در این پژوهش، دو هیدروژل کاپاکاراگینان با غلظت ۱/۵٪ و کاپاکاراگینان و موسیلاژ دانه به با نسبت ۱:۱ و غلظت نهایی ۱/۵٪ با کمک محلول کلرید پتاسیم ۵٪ تشکیل شدند. سپس توانایی پاسخ‌دهی این دو داربست به تغییر pH محیط مورد ارزیابی قرار گرفت و زنده‌مانی رده سلولی C2C12 در pH ۷/٤ و در هنگام تغییر pH از ۷/٤ به ۵/۵ بررسی شد. بررسی تغییر تورم با تغییر pH محیط (pH ۷-۱) نشان داد که برای داربست Carr بیشترین میزان تورم در pH ۵ و برابر با ۱٤۵٪ به دست آمد که با میزان تورم در سایر pH ها تفاوت معنی داری نشان داد (P<۰/۰۵). بیشترین میزان تورم برای داربست Carr:Quc نیز در pH ۵ و به میزان ٤۲۸٪ مشاهده شد که با میزان تورم در سایر pH ها تفاوت معنی داری نشان داد (P<۰/۰۵). همچنین تغییر تورم داربست‌ها با تغییر pH از ۷/٤ به ۵/۵ ارزیابی شد. داربست Carr هیچ تغییر تورمی در زمان‌های مورد مطالعه نشان نداد در حالی‌که تغییر تورم داربست Carr:Quc بعد از قرار گرفتن در pH ۵/۵ به مدت ۳۰، ٤۵، ٦۰، ۱۸۰ و ۳٦۰ دقیقه نسبت به pH ۷/٤ تفاوت معنی‌داری داشت (P<۰/۰۵). ارزیابی زنده‌مانی سلول‌های C2C12 نیز بر روی داربست Carr:Quc در شرایط نرمال و تغییر pH از ۷/٤ به ۵/۵ نشان داد که زنده‌مانی سلول‌ها در شرایط نرمال بیشتر بود. بررسی زنده‌مانی و رشد سلول‌ها در داربست Carr:Quc به مدت ۱۵ روز نشان داد که داربست مورد نظر سمیتی اعمال نکرد و سلول‌ها زنده‌مانی خود را در طول کشت حفظ کرده و در روزهای ۱٤ و ۱۵ کشت، تمایل تشکیل به توده‌های سلولی سه‌بعدی (اسفروئید) نشان دادند. یافته‌های این تحقیق می‌تواند راه‌گشای طراحی داربست‌های متشکل از بیوپلیمرهای خوراکی برای تسهیل مهندسی بافت گوشت آزمایشگاهی باشد.

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