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The Differences in Wetting Ability between Non-Crosslinked and Glutaraldehyde Crosslinked Chitosan-Gelatin-Hydroxiapatite Carbonate Composite Scaffolds

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Abstract

Wettability plays a key role in scaffold performance by supporting fluid interaction and nutrient diffusion. Chitosan-Gelatin-Carbonate Hydroxyapatite (C-G:CHA) scaffolds are highly biocompatible, yet glutaraldehyde crosslinking may alter pore structure and surface chemistry, thereby affecting the contact angle as an indicator of wetting ability. To analyze differences in surface wettability between non-crosslinked and 0.25% glutaraldehyde-crosslinked C-G:CHA scaffolds and to compare the 30:70 (w/w) and 40:60 (w/w) ratios. C-G:CHA scaffolds were synthesized using freeze-drying at ratios 30:70 and 40:60 and divided into non-crosslinked and crosslinked groups. Wettability was assessed using the sessile drop method with SBF and measured with a contact angle goniometer. Statistical analyses included Shapiro-Wilk, Levene's test, One-Way ANOVA, and Tukey HSD. The mean contact angles for non-crosslinked scaffolds were 64.833° (30:70) and 76.333° (40:60), increasing to 106.16° and 118.167° after crosslinking. ANOVA showed significant group differences ($p < 0.001$). Tukey HSD indicated significant differences among groups except between the crosslinked 30:70 (w/w) and 40:60 (w/w). Glutaraldehyde 0.25% crosslinking increases contact angle and reduces wetting ability. Significant differences were found between treatments and ratios, except between crosslinked 30:70 (w/w) and 40:60 (w/w) scaffolds.

Keywords: Wettability; Scaffold; Chitosan-Gelatin-Carbonate Hydroxyapatite; Glutaraldehyde 0;25%; Contact Angle

1. Introduction

Bone tissue engineering is a discipline that integrates biomaterial scaffolds, stem cells, and growth factors [1,2]. Scaffolds play a vital role as artificial extracellular matrices that provide structural support for tissue regeneration [3]. Chitosan and gelatin are chosen as biomaterials due to their antibacterial properties, biocompatibility, and ability to enhance cell adhesion without significant allergy risks [4,5]. This combination is enhanced with Hydroxyapatite Carbonate, which resembles human bone minerals to improve the bioactivity and hydrophilicity of the scaffold [6,7].

The ideal scaffold structure requires an average porosity of 150 μm to facilitate cell infiltration and nutrient diffusion, which are crucial for tissue survival [8]. In addition to biological aspects, mechanical strength in the form of compressive strength of 1-12 MPa is essential for the scaffold to mimic the characteristics of trabecular bone [9]. The use of crosslinking agents such as 0.25% glutaraldehyde has been shown to significantly increase stability and mechanical strength through the formation of amine bond. However, this crosslinking process is also known to reduce porosity and alter the surface properties of the material [10].

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This study focused on the C-G:CHA ratio of 30:70 (w/w) as a control due to its similarity to the Ca:P ratio in bone, as well as the 40:60 (w/w) ratio, which had previously shown similar characteristics. Although the mechanical strength of both ratios has been proven to meet minimum standards after the crosslinking process, their effect on water absorption capacity still needs to be further investigated [9,10]. Evaluation of wetting capacity is an important parameter to ensure that the scaffold remains stable and is not easily damaged when in contact with body fluids [11].

This study aims to compare the effect of 0.25% glutaraldehyde crosslinking treatment on the non-crosslinked group in terms of surface interaction with liquids [10]. The results of this study are expected to provide a comprehensive overview of the effectiveness of C-G:CHA scaffolds in supporting nutrient diffusion during the bone regeneration process [8].

2. Material and methods

2.1. Preparation of C-G:CHA Scaffolds

Chitosan–gelatin–carbonate hydroxyapatite (C-G:CHA) scaffolds were prepared at two ratios, such as 30:70 and 40:60 (w/w). For the 30:70 (w/w) ratio, 0.375 g chitosan, 0.375 g gelatin, and 1.75 g carbonate hydroxyapatite were used, whereas the 40:60 (w/w) ratio consisted of 0.5 g chitosan, 0.5 g gelatin, and 1.5 g carbonate hydroxyapatite. The formulation was adjusted proportionally to accommodate crosslinked and non-crosslinked groups. Gelatin was dissolved in 2 mL of 2% acetic acid and stirred at 50 °C until homogeneous. Carbonate hydroxyapatite was dispersed in 0.94 mL distilled water and subsequently mixed with the gelatin solution. Chitosan powder was added gradually under continuous stirring to form a uniform C-G:KHA gel. The gel was neutralized with 0.1 M NaOH, and the pH was adjusted to 7.0. The neutralized gel was cast into molds (5 × 5 × 0.5 cm), compacted to remove trapped air, frozen at −40 °C for 48 hours, and freeze-dried for 48 hours to obtain porous scaffolds.

2.2. Freeze-Drying Process

Scaffolds were freeze-dried using a condenser temperature of −40 °C, followed by vacuum drying for 48 hours, ensuring complete solvent sublimation and structural stabilization.

2.3. Crosslink Treatment

Scaffolds were rehydrated in 0.05 M acetic acid for 15 minutes, then immersed in 0.25% glutaraldehyde solution at 4 °C for 24 hours. Residual glutaraldehyde was removed by repeated washing with distilled water (five cycles, 10 minutes each). The scaffolds were subsequently frozen and freeze-dried for 24 hours each to obtain crosslinked samples [12, 13].

2.4. Wettability Analysis

Surface wettability was evaluated using a contact angle goniometer with the sessile drop technique. Scaffolds were placed on a glass substrate, and a 5 µL simulated body fluid (SBF) droplet was deposited on the surface. Droplet images were captured using a high-speed camera under visible light irradiation, and the contact angle was measured using image analysis software [12].

2.5. Statistical Analysis

Data were analyzed using the Shapiro–Wilk test for normality and Levene's test for homogeneity of variance. Differences in wettability among groups were evaluated using one-way ANOVA, followed by Tukey's HSD post-hoc test, with statistical significance set at $p < 0.05$.

3. Results and discussion

3.1. Scaffold Synthesis

C–G:CHA scaffolds were successfully synthesized at 30:70 and 40:60 (w/w) ratios using a deep freezing process followed by freeze drying. The scaffolds exhibited uniform square geometry with dimensions of 5 × 5 × 0.5 cm. Subsequently, selected samples were crosslinked using 0.25% glutaraldehyde. Macroscopically, crosslinked scaffolds appeared denser and more compact than non-crosslinked scaffolds, indicating structural modification due to the crosslinking process.

3.2. Surface Wettability of C-G:CHA

Surface wettability was evaluated by contact angle measurements, which were performed six times for each ratio, consisting of three measurements for non-crosslinked and three for crosslinked samples. Contact angle measurement is considered one of the most reliable methods for evaluating the wettability of solid surfaces by assessing the interaction between liquid droplets and solid substrates [14].

Non-crosslinked scaffolds exhibited contact angle values below 90° at both ratios (64.83° for 30:70 and 76.33° for 40:60), indicating hydrophilic surface characteristics. Hydrophilic surfaces are favorable for fluid absorption and cellular attachment, which are essential for tissue regeneration applications [15]. In contrast, scaffolds crosslinked with 0.25% glutaraldehyde showed a significant increase in contact angle values above 90°, reaching 106.16° and 118.17° for the 30:70 and 40:60 ratios, respectively, indicating a shift toward hydrophobic behavior.

Statistical analysis using the Post Hoc Tukey test revealed significant differences between non-crosslinked and crosslinked scaffolds at both ratios ($p < 0.05$). However, no significant differences were observed between the 30:70 and 40:60 ratios within the same treatment groups.

3.3. Effects of Crosslinking on Wettability Behaviour

The increased contact angle observed after glutaraldehyde crosslinking indicates reduced surface wettability. This phenomenon can be attributed to the formation of a denser and more rigid polymer network, which reduces surface free energy and limits the interaction between scaffold surfaces and water molecules [12,13]. Additionally, crosslinking reduces scaffold porosity and surface roughness, thereby restricting liquid penetration into the internal pore structure [16].

Surface wettability is strongly influenced by surface roughness, porosity, surface tension, and viscosity of the contacting liquid [17]. Rough and porous surfaces tend to enhance liquid absorption, resulting in lower contact angle values, whereas smoother and more compact surfaces exhibit higher contact angles [18]. Therefore, the increased contact angle observed in crosslinked scaffolds is consistent with the reduced pore size and increased structural compactness induced by glutaraldehyde treatment.

Non-crosslinked C–G:CHA scaffolds retained hydrophilic properties, which promote fluid absorption, nutrient diffusion, and cell adhesion. Conversely, crosslinked scaffolds exhibited reduced liquid absorption capacity due to decreased pore volume, although this structural modification contributes positively to enhanced mechanical strength and reduced degradation rates [9,19].

3.4. Implications for Bone Tissue Engineering

The freeze-drying technique employed in this study generated interconnected porous structures that are essential for nutrient diffusion and cellular infiltration [20]. Previous studies have reported that pore sizes around 150 μm are optimal for bone tissue engineering, as they facilitate cell adhesion, proliferation, and osteogenic differentiation. Although glutaraldehyde crosslinking reduced surface wettability and liquid absorption, crosslinked K–G:CHA scaffolds demonstrated improved structural integrity and mechanical stability. Therefore, scaffold selection should be tailored to the intended application, balancing wettability and mechanical performance. Non-crosslinked scaffolds are more suitable for applications requiring high fluid absorption and bioactivity, whereas crosslinked scaffolds are advantageous when enhanced mechanical strength and slower degradation are required for bone tissue engineering applications [15].

4. Conclusion

Based on the results of the study, it can be seen that cross-linking in the C-G:CHA series produces higher contact angle values compared to the non-cross-linked series. The C-G:CHA scaffold with a 40:60 (w/w) ratio produced higher contact angle values compared to the 30:70 (w/w) ratio, both in cross-linked and non-cross-linked scaffolds.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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