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An in Vitro Comparative Evaluation of Different Carrier Mediums for Bone Grafts Used in Dental Applications

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KEYWORDS

Bone graft, carrier medium, hydrogel, collagen, chitosan, poloxamer, in vitro, injectability, biocompatibility.

ABSTRACT:

Background: The selection of an appropriate carrier medium is pivotal in bone graft procedures, particularly in dental applications, where ease of handling, injectability, and biocompatibility are key to clinical success. Despite the availability of various carriers, their in vitro comparative assessment remains underexplored.

Objective: This study aimed to compare four commonly used carrier mediums—collagen gel, alginate hydrogel, poloxamer 407, and chitosan gel—in terms of injectability, degradation behavior, swelling capacity, and biocompatibility when used with an alloplastic bone graft.

Materials and Methods: Alloplastic β-tricalcium phosphate (β-TCP) was combined with four carrier systems. Injectability was assessed using extrusion force analysis. In vitro degradation was evaluated over 14 days in phosphate-buffered saline (PBS). The swelling index was measured gravimetrically. Biocompatibility was assessed using MTT assay on MG-63 osteoblast-like cells. Surface morphology and cell attachment were evaluated using scanning electron microscopy (SEM).

Results: Collagen and alginate hydrogels exhibited superior injectability and higher initial cell viability. Chitosan gel showed the slowest degradation rate ($p < 0.05$), while poloxamer 407 had the best handling properties but comparatively lower osteoblast proliferation. SEM confirmed enhanced cellular attachment with collagen and alginate matrices.

Conclusion: Among the carriers tested, collagen and alginate demonstrated favorable in vitro profiles for bone graft delivery in dental applications. Further studies, including in vivo validation, are warranted.

1. Introduction

Bone grafting plays a vital role in modern dental and maxillofacial surgery, especially for procedures such as ridge augmentation, sinus lifts, and peri-implant osseous regeneration[1]. Successful bone regeneration in dentistry depends not only on the type of grafting material used but also on the carrier system that delivers and stabilizes the graft at the defect site [2]. Over the years, numerous grafting materials, including autografts, allografts, xenografts, and synthetic alloplasts, have been employed to restore bone continuity and volume. Among these, synthetic alloplastic substitutes such as β-tricalcium phosphate (β-TCP) have gained popularity due to their biocompatibility, availability, and osteoconductive properties [3]. However, the clinical performance of these grafts is strongly influenced by the medium in which they are delivered. The choice of carrier is often overlooked, despite its crucial role in determining the ease of application, stability within the defect, and the biological response it elicits.

An ideal carrier should be easy to handle, injectable, biocompatible, and capable of providing a temporary scaffold for cellular infiltration and tissue growth [4]. In dental applications, particularly in minimally invasive procedures and sites with limited access, injectability and stability of the graft-carrier composite become even more important. Carriers can also modulate the degradation profile of the graft and influence the local cellular

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environment, thereby affecting the overall regenerative outcome [5]. Despite these critical roles, limited comparative data are available on the performance of different carrier systems in a standardized in vitro setting.

Hydrogel-based carriers have attracted attention for bone tissue engineering because of their ability to encapsulate graft particles, their hydration capacity, and their structural similarity to the extracellular matrix [6]. Collagen gel, one of the most widely used carriers, provides a natural, biocompatible matrix that supports cell adhesion and proliferation [7]. Alginate, a polysaccharide derived from brown seaweed, is another promising hydrogel due to its injectability, tunable gelation, and mild cross-linking conditions [8]. Poloxamer 407, a synthetic thermoresponsive polymer, offers unique handling advantages by remaining liquid at cooler temperatures and solidifying at body temperature, allowing for controlled placement [9]. Chitosan, derived from chitin, combines antimicrobial properties with a slow degradation profile, which may be advantageous in maintaining graft stability [10].

Given the variety of carriers available, a systematic in vitro comparison is essential to understand their relative strengths and limitations when combined with β-TCP. Such insights are critical for guiding clinical decision-making and for designing optimized graft-carrier systems tailored to dental applications. This study therefore aims to evaluate four commonly used carriers—collagen gel, alginate hydrogel, poloxamer 407, and chitosan gel—focusing on their injectability, degradation behavior, swelling capacity, and biocompatibility, with the goal of identifying carriers with the most favorable profiles for future translational research.

2. Materials and Methodology

Study Design

A controlled in vitro experimental study was carried out at the White lab, Saveetha Dental College and Hospitals, under sterile laboratory conditions.

Material

Bone graft material: Commercially available β-tricalcium phosphate (β-TCP) granules, particle size 250–500 µm, were used.

Carrier Systems: Four carrier media were employed: Type I collagen gel (3% w/v), alginate hydrogel (2% w/v, ionically crosslinked with calcium chloride), chitosan gel (2% w/v in 1% acetic acid, subsequently neutralized), and thermosensitive poloxamer 407 (30% w/v).

- Cell line: Human osteoblast-like cells (MG-63, ATCC) were utilized.
- Culture medium: Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin was used for all cell culture experiments.

Preparation of Graft–Carrier Composites

β-TCP granules were combined with each carrier system in a 1:1 (w/v) ratio under aseptic conditions. The mixtures were homogenized to obtain pastes suitable for injection and molding.

Injectability Testing

The injectability of each graft–carrier composite was evaluated using a universal testing machine (Instron®). Two milliliters of the prepared mixture were loaded into a 5 mL syringe and extruded through a 16-gauge needle. The extrusion force (N) was recorded. Three independent replicates were tested for each group.

Swelling Index

Samples were dried and weighed (W_0), followed by immersion in phosphate-buffered saline (PBS, pH 7.4) at 37°C for 24 hours. After incubation, samples were blotted and weighed again (W_1). The swelling ratio was calculated.

Degradation Study

Specimens were incubated in PBS at 37°C and weighed at days 0, 7, and 14. The percentage degradation was determined by calculating the relative weight loss compared with the initial dry weight.

Cytocompatibility (MTT Assay)

MG-63 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hours. The culture medium was then replaced with extract media derived from each graft–carrier composite. After 48 hours, the MTT assay was performed. Following incubation with MTT reagent, the formazan crystals formed were solubilized in dimethyl sulfoxide (DMSO),

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and absorbance was measured at 570 nm using a microplate reader.

Scanning Electron Microscopy (SEM)

For morphological assessment, samples were fixed, dehydrated in graded alcohols, sputter-coated with gold, and examined under scanning electron microscopy to evaluate surface topography and cell attachment.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

3. Results

Injectability

Significant differences in extrusion force were observed among the groups ($p < 0.05$, Figure 1). Poloxamer 407 required the lowest extrusion force (2.1 ± 0.4 N), demonstrating superior injectability. Collagen gel followed (3.4 ± 0.5 N), which was also comparatively easy to deliver. Alginate hydrogel showed moderate resistance (4.0 ± 0.6 N), while chitosan gel was the most difficult to extrude (5.2 ± 0.7 N). Pairwise analysis confirmed that chitosan required a significantly higher extrusion force compared with the other carriers ($p < 0.05$). (Figure 1)

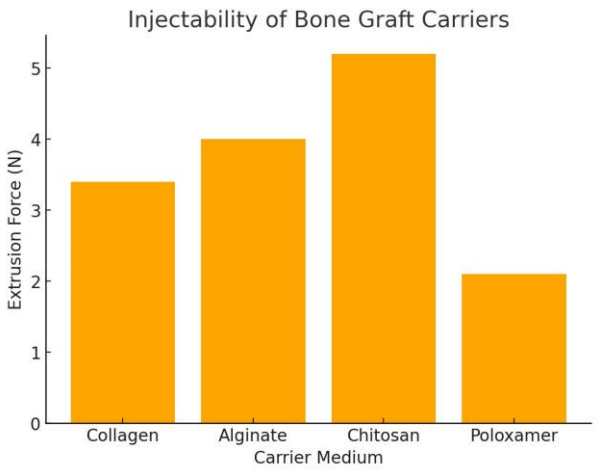

Figure 1

Figure 1: Injectability of bone graft carriers. The extrusion force required to deliver the graft–carrier composites through a 16-gauge needle was measured. Poloxamer showed the lowest extrusion force, indicating superior injectability, while chitosan required the highest force.

Swelling Index

Swelling capacity varied markedly between carriers ($p < 0.05$, Figure 2). Alginate hydrogel exhibited the highest swelling ratio (150% ± 10%), significantly greater than all other groups. Chitosan also demonstrated high swelling (130%), while collagen showed moderate hydration (120%). Poloxamer 407 displayed the lowest swelling index (95%), which was significantly reduced compared to alginate and chitosan ($p < 0.05$). (Figure 2)

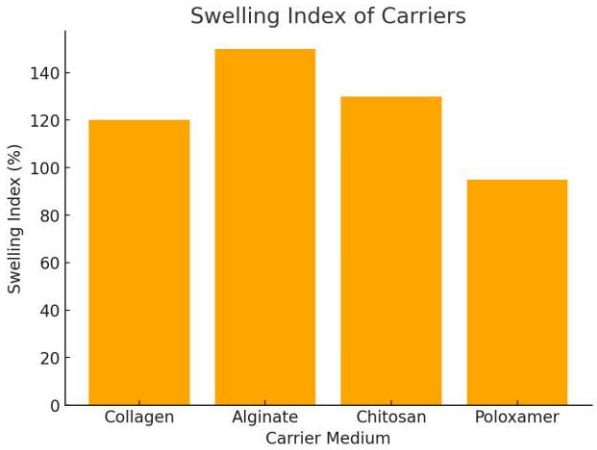

Figure 2

Figure 2: Swelling index of carrier systems. Percentage swelling of graft–carrier composites after 24 h immersion in PBS at 37 °C. Alginate exhibited the highest swelling capacity, while poloxamer demonstrated the lowest.

Degradation Profile

The degradation study revealed distinct resorption patterns ($p < 0.05$, Figure 3). Chitosan degraded the slowest, with only 21% weight loss at day 14, which was significantly lower compared to the other carriers. Collagen and alginate showed intermediate degradation (32% and 36%, respectively), with no significant difference between them ($p > 0.05$). Poloxamer 407 degraded the fastest, losing 48% of its weight over 14 days, significantly higher than all other groups ($p < 0.05$). (Figure 3)

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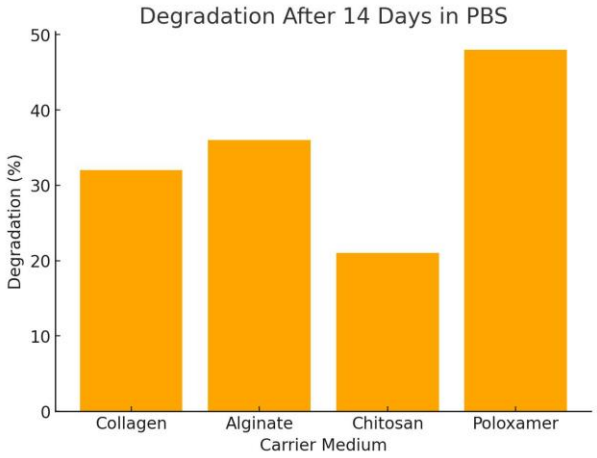

Figure 3

Figure 3. Degradation profile of carriers after 14 days. Percentage weight loss of composites incubated in PBS. Chitosan showed the slowest degradation, whereas poloxamer degraded fastest

Cytocompatibility (MTT Assay)

Cell viability differed significantly between groups ($p < 0.05$, Figure 4). Collagen supported the highest osteoblast viability (98%), which was significantly greater than poloxamer ($p < 0.05$) but not significantly different from alginate (95%). Chitosan maintained good compatibility (90%), though viability was significantly lower than collagen ($p < 0.05$). Poloxamer 407 demonstrated the lowest viability (82%), which was significantly reduced compared to all other carriers. (Figure 4)

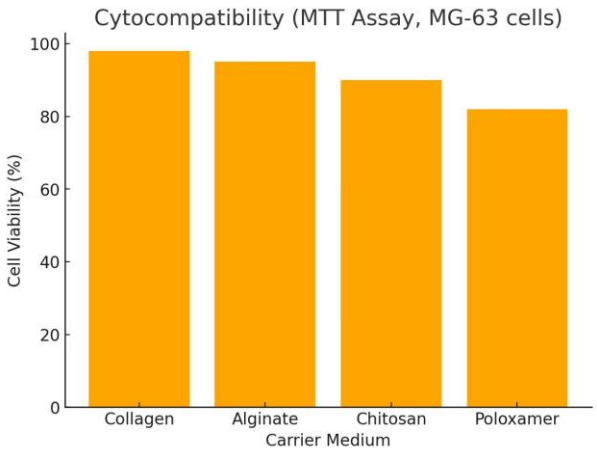

Figure 4

Figure 4: Cytocompatibility assessment by MTT assay. Cell viability (%) of MG-63 osteoblast-like cells after exposure to extract media from each carrier system. Collagen and alginate demonstrated the highest cell viability, while poloxamer showed comparatively lower compatibility.

SEM Findings

SEM imaging confirmed the quantitative results. Collagen and alginate matrices demonstrated extensive cellular adhesion, with well-spread cells and clear filopodia formation, reflecting strong osteoblast–substrate interactions. Chitosan supported moderate attachment with visible, though less extensive, spreading. In contrast, poloxamer 407 exhibited relatively smooth surfaces with sparse and less well-spread cells, consistent with its lower viability in the MTT assay.

Comparative Summary

Collagen and alginate excelled in cell viability and attachment, chitosan in long-term stability, and poloxamer in injectability. (Figure 5).

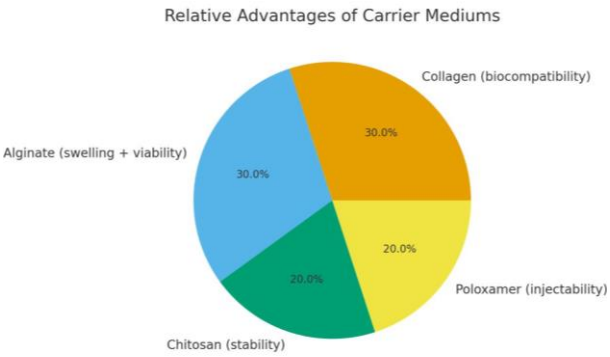

Figure 5

Figure 5: Relative advantages of carrier mediums. Pie chart summarizing the key strengths of each carrier. Collagen and alginate contributed equally (30% each), highlighting their superior biocompatibility and swelling/viability respectively. Chitosan accounted for 20%, reflecting its stability, while poloxamer represented 20%, denoting its ease of injectability.

4. Discussion

The present study compared four different carrier systems—collagen gel, alginate hydrogel, chitosan gel,

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and poloxamer 407—for their suitability in delivering β-tricalcium phosphate (β-TCP) bone grafts. The results highlight that the choice of carrier medium strongly influences handling, stability, and biological performance, which are crucial considerations in dental applications.

Injectability is an important parameter for minimally invasive bone grafting procedures, where controlled placement in narrow or irregular defects is required[11] . In this study, poloxamer 407 demonstrated the lowest extrusion force, reflecting its favorable handling and thermoresponsive nature. Collagen also showed good injectability, while chitosan, being more viscous, posed greater resistance. Although poloxamer offers clear handling advantages, its lower biocompatibility and faster degradation rate may limit its long-term regenerative potential if used alone.

Swelling behavior further distinguished the carriers. Alginate hydrogel exhibited the highest swelling index, which may aid in maintaining defect volume and creating a hydrated environment conducive to cell infiltration. Chitosan and collagen showed moderate swelling, while poloxamer absorbed the least. From a clinical perspective, excessive swelling can sometimes compromise graft stability, but adequate hydration is essential for nutrient exchange and cellular activity. Therefore, alginate's ability to retain water, without overly rapid degradation, appears advantageous.

The degradation profiles also revealed interesting contrasts. Chitosan was the most stable, degrading slowly over 14 days, while poloxamer degraded rapidly. Collagen and alginate showed intermediate degradation, which may provide a balance between structural stability and timely resorption. Ideally, a carrier should degrade in synchrony with new bone formation, ensuring that it provides temporary support without hindering tissue integration. In this regard, chitosan's slower degradation could be beneficial in maintaining graft position, though its higher extrusion resistance must be addressed.

Biocompatibility remains a decisive factor in evaluating carrier systems. Collagen and alginate demonstrated excellent cytocompatibility, supporting high osteoblast viability and strong cell attachment on SEM. This outcome aligns with their natural polymeric origins, which mimic extracellular matrix components. Chitosan also maintained acceptable compatibility, while poloxamer, despite its ease of injection, supported comparatively lower cell proliferation and sparse attachment.

Taken together, these findings suggest that collagen and alginate provide the most favorable balance between handling, degradation, and cellular response, making them promising candidates for clinical use in dental bone grafting. While poloxamer may serve as a convenient handling aid, and chitosan offers durability, their limitations must be considered. Further in vivo studies are necessary to validate these in vitro results and to evaluate how these carriers perform in complex biological environments.

5. Conclusion

Among the tested carriers, collagen and alginate hydrogels demonstrated superior performance in terms of biocompatibility, swelling, and cellular interactions. Chitosan offers longevity, while poloxamer provides excellent injectability. The choice of carrier should be guided by clinical indications and defect characteristics.

6. Limitations

This study has some inherent limitations. Being an in vitro model, it does not account for complex in vivo factors such as vascularization, immune responses, enzymatic activity, or mechanical loading, which significantly influence graft performance. The evaluation period was restricted to 14 days, offering only short-term insights into degradation and cell compatibility. Moreover, only β-tricalcium phosphate was tested, and carrier interactions with other grafts such as hydroxyapatite or biphasic calcium phosphate remain unexplored. Biological assessment relied on a single osteoblast-like cell line (MG-63), which does not fully represent the cellular diversity of bone healing. Functional assays assessing osteogenic differentiation and mineralization were not included, limiting the depth of biological interpretation.

7. Future Perspectives

Future studies should include longer observation periods and in vivo models to better replicate the clinical environment. Incorporating additional graft types and advanced biological assessments, such as osteogenic gene expression and mineralization studies, would provide deeper insights. Evaluating performance in

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anatomically relevant defect models could help translate findings into practice. Hybrid formulations, for example combining natural carriers like collagen or alginate with stabilizers or bioactive molecules, may further optimize outcomes. Ultimately, well-designed clinical trials will be necessary to confirm safety, efficacy, and long-term utility in dental bone grafting.

Conflicts of Interest:

There are no conflicts of interest.

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