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Evaluation of graphene oxide-doped poly-lactic-co-glycolic acid (GO-PLGA) nanofiber absorbable plates and titanium plates for bone stability and healing in mandibular corpus fractures: An experimental study



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KEYWORDS
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Abstract *Background:* Open reduction with internal fixation is the preferred treatment option for displaced facial bone fractures. The superior mechanical properties of metallic plates have made them the most widely used material in existing bone fixation systems. However, after the healing period, these permanent plates can cause various problems. Alternative bioresorbable materials are being investigated to reduce these potential problems. This study compares bone stability and viability by using graphene oxide (GO)-doped poly-lactic-co-glycolic acid (PLGA) nanofiber plates and titanium plates for rats with fractured mandibles.

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Materials and methods: The study included 20 male Sprague-Dawley rats, divided into four groups: a control group (Group I), a mandibular fracture group with no additional application (Group II), a mandibular fracture group repaired with titanium plates (Group III), and a mandibular fracture group repaired with GO-PLGA plates (Group IV). After 2 months, all of the rats were euthanized. A bone compression test was performed to assess bone stability, and a histological examination was performed to evaluate bone healing.
Results: The osteocyte lacunae, Haversian ducts, canaliculi, and vascular structures of Group IV were found to be higher. In the compression test, vertical compression was applied to the bone axis, and Group IV had a higher maximum load and maximum stretch. GO-PLGA plates were found to be statistically superior to titanium plates in terms of both bone stability and bone healing ($p < 0.05$).
Conclusions: The present study found that GO-PLGA plates are more effective than titanium plates for the treatment of mandibular corpus fractures.
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The treatment of mandibular fractures represents a major challenge in plastic and reconstructive surgery due to complex anatomy of the mandible and the high risk of complications.[1,2] Currently, the use of titanium plates and screws is the gold standard for the fixation of mandibular fractures,[3] but there is growing interest in the development of new biomaterials that can improve healing and reduce the risk of complications.[4] Graphene oxide poly(lactic-co-glycolic acid) (GO-PLGA) composite materials have shown great promise in this regard, due to their unique mechanical and biological properties.[5,6] In this study, we aimed to evaluate the effectiveness of GO-PLGA plates and screws in treatment of mandibular fractures in a rat model. Specifically, we compared biomechanical and histological properties of mandibular fractures treated with GO-PLGA plates and screws to those treated with traditional titanium plates and screws.[6,7] Our hypothesis was that GO-PLGA composite materials would provide superior biomechanical and histological outcomes compared to titanium.[8,9]

Materials and methods

Ethics committee

Our study was approved by Bagcılar training and research hospital's ethics committee (number 2020-51). All surgical procedures were performed in accordance with the guidelines for the care of laboratory animals published by the United States National Institutes of Health.[10]

Animal model

The study included 20 male Sprague-Dawley rats with an average weight of 300 g. The rats were housed in cages at a temperature of 20–22 °C with a 12-h light and dark cycle before the surgical intervention. They were watered and fed ad libitum. The treated area was monitored twice a day for adhesion, discharge, redness, and swelling. Behavior, activity, and posture control were performed at same frequency. A loss of weight of more than 20% of the body weight was determined as criterion for an animal to be excluded from the experimental protocol.

Sample size estimation and the determination of groups

Using the power analysis program (v. 3.1.9.2), optimum group size and number of rats were determined with reference to previous studies.[11,12] While determining the sample size, a type 1 error was referenced as 0.05 and a type 2 error was referenced as 0.2.

The rats were divided into four groups with five rats in each group. Group I was control group; in Group II, a mandible fracture was created and no operation was performed; in Group III, the fractures were repaired with titanium plates after they were created; and in Group IV, the fractures were repaired with GO-PLGA plates after they were created.

Manufacturing GO-PLGA plates and screws

Before the experiment, the GO-PLGA plates used in the study were produced by Red Medikal (Istanbul, Turkey). A suspension of PLGA (15% by weight) mixed with 1% GO was prepared by placing 30 mg of GO into 20 mL of tetrahydrofuran/dimethyl formamide mixture solvent (3/1, v/v) and then applying vigorous magnetic stirring to form a homogeneous solution. Then, 3 g of PLGA was added to this solution at 4 °C for 24 h, and the solution was subjected to a water bath ultrasonic cleaner (50 W) for 10 min before use. The PLGA/GO suspension was separated by centrifugation at 4500 rpm for 45 min, and 5-mm double-hole GO-PLGA plates and 1-mm diameter GO-PLGA screws were dried at 45 °C for 12 h by means of special molds prepared using a lyophilizer. The titanium plates and GO-PLGA plates were sterilized with ethylene oxide before use in the experimental animals.

Surgical method

All surgical procedures were performed by the same surgeon (CU). A surgical loop with five times magnification

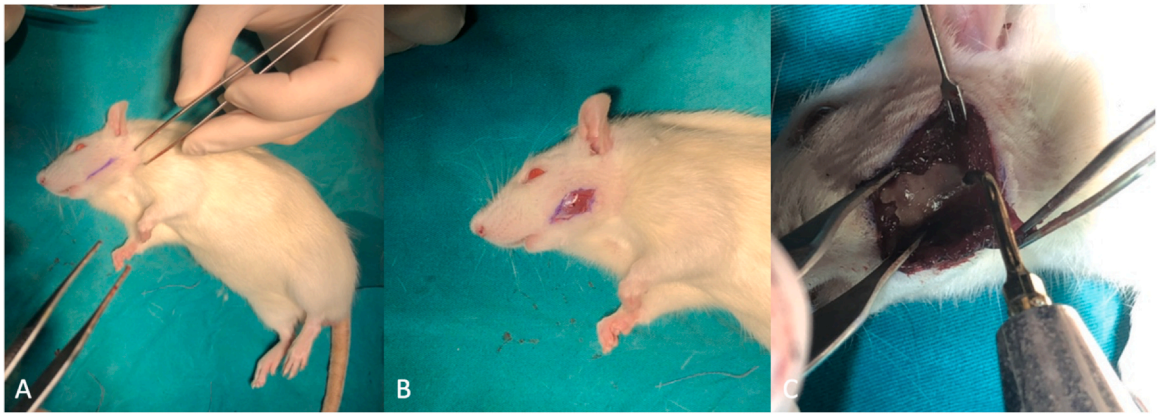


Figure 1 Performing the surgical procedure. **A:** Rats were placed in the supine position. **B:** The masseter muscle was dissected, mental nerve was preserved, and subperiosteal plane was reached. **C:** Bicortical vertical osteotomy was performed 5 mm posterior to the third molar tooth with a Piezo device (Woodpecker, China) with a 2-mm sharp tip.

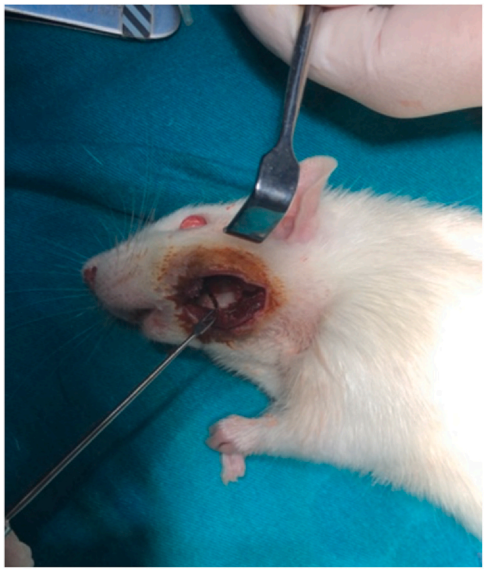


Figure 2 The bone segments were repositioned and no additional intervention was made in Group II.

(033-OBO 4X Carl Zeiss, Germany) and standard microsurgical instruments were used.

Group III and IV rats were placed in the supine position under appropriate intraperitoneal anesthesia (intraperitoneal 60 mg/kg ketamine hydrochloride [Ketalar Pfizer Warner-Lambert, Turkey] and 10 mg/kg xylazine [Alfazyne, Alfasan International BV, Netherlands]) and a pad was placed underneath to reduce heat loss (Figure 1A). Finger pinch responses and jaw or skeletal muscle tone were monitored every 5 min to monitor the depth of anesthesia. The left mandibular areas were shaved, and surgical area was cleaned with povidone iodine. After a submandibular incision 2 cm into the left mandible, the masseter muscle was dissected, the mental nerve was preserved, and the subperiosteal plane was reached (Figure 1B). A bicortical vertical osteotomy was performed 5 mm posterior to the third molar tooth with a Piezo device (Woodpecker, China) with a 2-mm sharp tip (Figure 1C). Then the bone segments were repositioned and no additional intervention was made in Group II (Figure 2). In Group III, they were fixed with a 5-mm two-hole titanium plate and two 1-mm diameter titanium screws (Figure 3). In Group IV, a 5-mm two-hole GO-PLGA plate was fixed with two 1-mm diameter GO-PLGA screws (Figure 4).

After these procedures, the bleeding was controlled, the subcutaneous tissue was sutured with 4/0 polyglactin sutures, the skin was sutured with 3/0 silk, and the operation was terminated.

Euthanasia of the rats

At 8 weeks, the rats were euthanized using a high dose of anesthetic (IV administration of pentobarbital). After euthanasia, the mandibles of the rats were excised. The bones of the rats in the groups were sampled en bloc for the biomechanical test, while for histological tests, specimens were collected with 3 mm proximal and distal portions of the fracture line included.

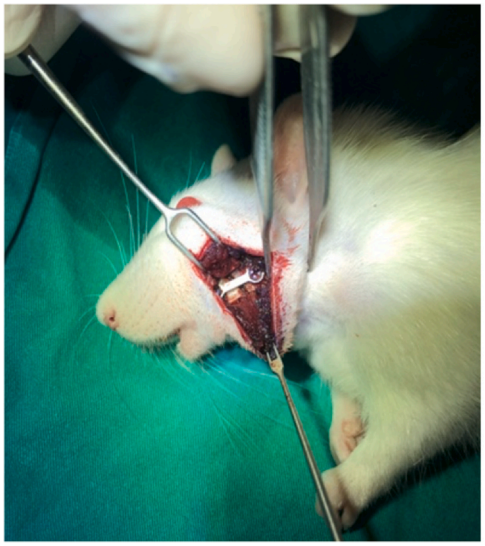


Figure 3 In Group III, the bone segments were fixed with a 5-mm two-hole titanium plate and two 1-mm diameter titanium screws.

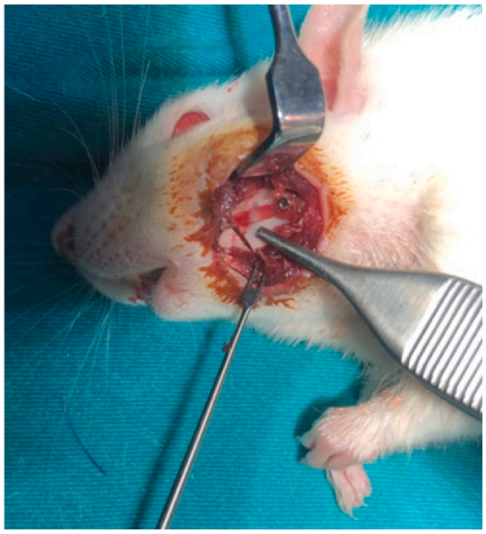


Figure 4 In Group IV, a 5-mm two-hole GO-PLGA plate was fixed with two 1-mm diameter GO-PLGA screws.

Biomechanical and histological tests after euthanasia

A bone compression test was used as the biomechanical test. Two mandible samples from each group were subjected to biomechanical testing. Vertical compression was applied to the bone axis, and the universal compression test (Lloyd instruments) was used. (At a pressure of 14 Mpa (MegaPascal) for a duration of 1 min).[13] (Figure 5). The speed of the load was set to 5 mm/min. The results were transferred to the computer. The physical endurance of the bone tissue was measured by the compression test.

For the histopathological-immunohistochemical study, three samples from each of the experimental animals in each of the groups were transferred to the fixative to be analyzed by histochemical methods. They were blocked after routine tissue follow-up procedures. Sections taken from the blocks were stained with histochemical and immunohistochemical dyes and examined under the light microscope. Bone strength tests were also performed. The histopathological-immunohistochemical evaluation showed the viability of osteocyte lacunae, Haversian channels, canaliculi, and vascular structures. The mandible specimens were examined using a dissecting microscope. The sections were approximately 5 mm wide and buffered in 10% neutral formaldehyde (Merck). They were labeled according to the groups. The osteoblastic activation within the marked Haversian channels was automatically counted by the system, and the number of marked areas in square micrometers was determined. Two ratios were calculated. The ratio of osteocytes to lacunae is the ratio of the number of lacunae containing osteocytes to the total number of lacunae. The Haversian canal ratio is the ratio of the number of Haversian canals containing veins to the total number of Haversian canals.



Figure 5 Bone compression test.

Statistical analysis

Data analysis was performed using SPSS (IBM SPSS Statistics for Mac, Version 21.0, IBM Corp.). Mean, standard deviation, median, and interquartile range values were used for descriptive statistics. Kruskal-Wallis test was used for ordinal data and analysis of variance test was used for continuous parameters. A p value of < 0.05 was considered statistically significant.

Results

None of the experimental animals required exclusion from the experimental protocol, and none of the 20 rats were lost during the study.

When the number of lacunae containing osteocytes and the total number of lacunae in the groups were compared, the total number of lacunae and lacunae containing osteocytes in Group I was significantly higher than those in the other groups. The total number of lacunae and lacunae containing osteocytes in Group IV was significantly higher than in Groups II and III. The total number of lacunae and lacunae containing osteocytes in Group III was significantly higher than in Group II ($p < 0.05$). When the ratio of the number of lacunae containing osteocytes to the total number of lacunae was calculated as a percentage, the percentage value in Group II was significantly lower than that of the other groups ($p < 0.05$), but there was no significant difference between the other three groups ($p > 0.05$) (Figure 6).

When the number of Haversian canals containing vessels and the total number of Haversian canals in the groups was compared, the total number of Haversian canals and vessel-containing Haversian canals in Group I was significantly higher than those in the other groups ($p < 0.05$). The total number of Haversian canals and vessel-containing Haversian canals in Group IV was significantly higher than those in Groups II and III ($p < 0.05$). The total number of Haversian canals and vessel-containing Haversian canals in Group 3 was significantly higher than that in Group II ($p < 0.05$). When the ratio of the number of Haversian canals containing vessels to the total number of Haversian canals was calculated as a percentage, it was determined that the percent in Group IV was significantly higher than those of the other three groups ($p < 0.05$) (Figure 6).

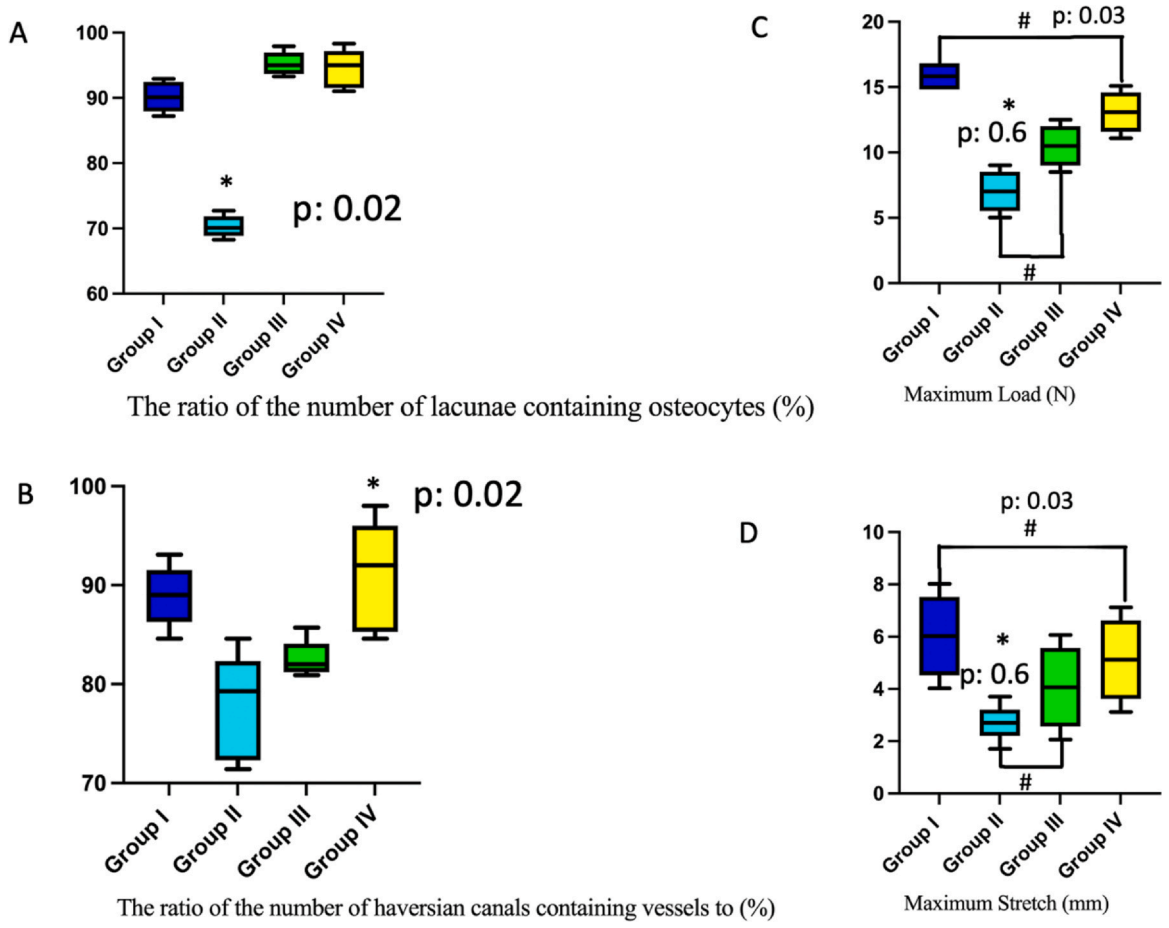


Figure 6 Graphs of histopathological results, maximum load, and stretch results. **A:** Percentage of the ratio of the number of lacunae containing osteocytes to the total number of lacunae (%) (*:*Group II was significantly lower than the other groups. p value:0.02*). **B:** Percentage of the ratio of the number of Haversian canals containing vessels to the total number of Haversian canals (%)(*:*The percent value in Group IV was significantly higher than the other three groups p value:0.02*). **C:** Maximum Load *(#: The values in Group I and Group IV were significantly higher than the other two groups. p value:0.03 *: There was no significant difference between Group I and Group IV, and between Groups II and III. p value:0.6)*. **D:** Maximum Stretch *(#: The values in Group I and Group IV were significantly higher than the other two groups. p value:0.03 *: There was no significant difference between Group I and Group IV, and between Groups II and III. p value:0.6)*.

During histological examination, a detailed observation of osteoclastic activity was conducted, particularly in specific areas. Under the microscope, the number and density of osteoclastic cells were assessed, and Howship's lacunae on the bone surface around osteoclastic cells were examined. The presence of these lacunae may indicate the occurrence of bone resorption by osteoclasts. Additionally, focusing on vascular changes associated with osteoclastic activity, areas where blood vessels dilated or increased in number were identified. Various changes in bone tissue were also scrutinized under the microscope; for instance, cracks, voids, or other morphological alterations that could be linked to osteoclastic activity.[13]

These evaluations based on these criteria allowed for a more detailed and comprehensive description of the differences in osteoclastic activity among groups. These observations provide a crucial insight into how osteoclastic activity may vary across groups.

This is in conjunction with the observation that the mandibular corpus in Group I exhibited an osteoblastic distribution, with no alterations observed in osteocytes or other histological structures.[13] In Group II, a fracture site was identified in the mandibular corpus structure. Notably, compared to Group I, Group II displayed pronounced osteoclastic activity, characterized by the presence of Howship's lacunae in extensive regions and a high density of osteoclastic cells. Furthermore, an increase in the number and dilation of vessels was noted in the fractured area. Similar findings were observed in the mandibular corpus structure of Group III, as illustrated in Figure 7.

When the maximum load and stretch values of the bones in the groups were compared, it was seen that the values in Group I and Group IV were significantly higher than those in the other two groups ($p < 0.05$). There was no significant difference between Group I and Group IV, and between Groups II and III ($p > 0.05$) (Figure 6).

Discussion

Titanium plates are commonly used in maxillofacial trauma because of their low density, nontoxicity, low modulus of

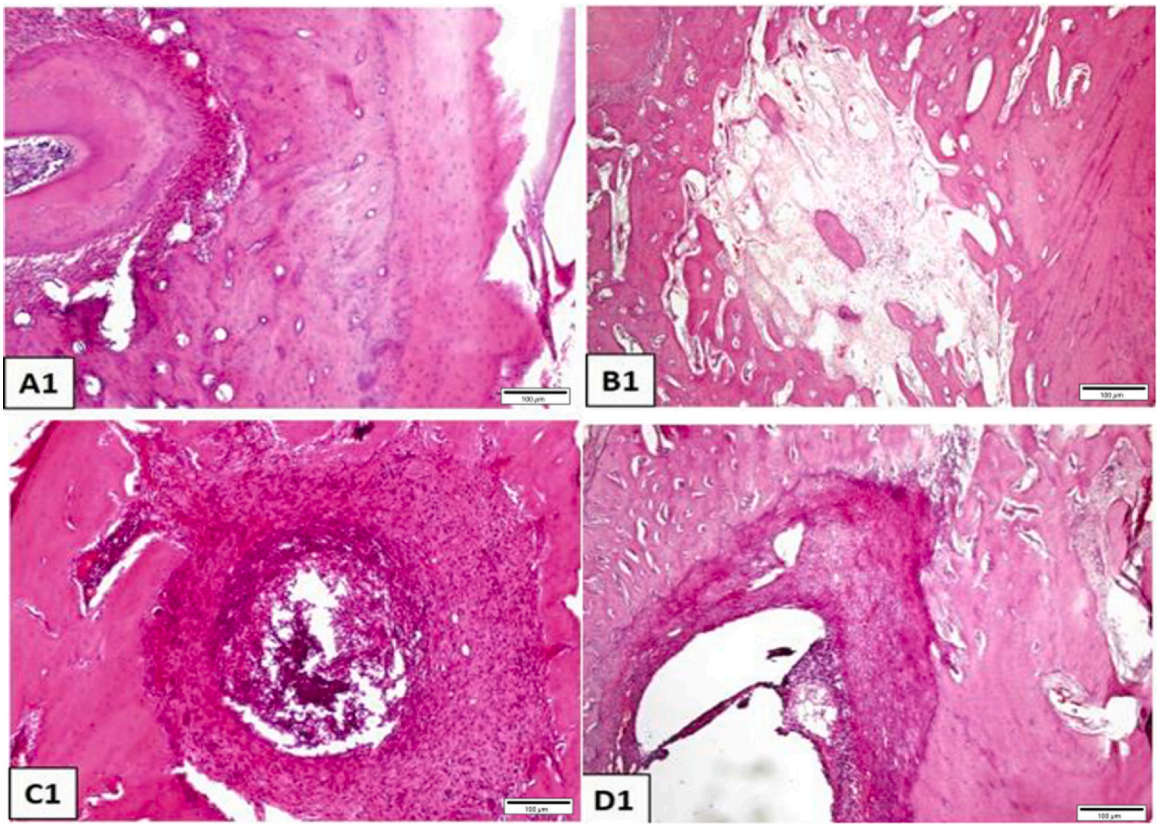


Figure 7 Histological features of the groups. **A1:** Group I Hematoxylin eosin staining (4× magnification). **B1:** Group II Hematoxylin eosin staining (4× magnification). **C1:** Group III Hematoxylin eosin staining (4 × magnification). **D1:** Group IV Hematoxylin eosin staining (4× magnification).

elasticity, and ease of shaping at room temperature.[5,6] However, patients often experience long-term complications, such as infection, plate exposure, cold sensitivity, and palpation pain.[14,15] GO-PLGA nanofiber absorbable plates contribute significantly to bone ossification, enhancing the tension and load resistance.[16] They offer superior long-term outcomes compared to titanium plates, effectively addressing complications. Despite being in the experimental stage, the anticipated widespread use of absorbable GO-PLGA nanofiber plates is promising.[17] However, potential contradictions may arise due to GO characteristics.

We compared the efficacy of GO-PLGA plates and screws with titanium plates and screws in fixing mandibular fractures in rats. Our results showed that GO-PLGA plates and screws were more effective than titanium plates and screws in promoting bone healing and improving the biomechanical properties of the mandible. The histological analysis revealed that the repair process was more advanced in the GO-PLGA group, with increased osteocyte maturation and calcification compared to that in the titanium group. Furthermore, the number of Haversian canals containing vessels was significantly higher in the GO-PLGA group, indicating improved vascularity and blood supply to the repaired area. These findings are consistent with those of previous studies that reported the potential advantages of biodegradable polymers over titanium in fracture fixation. For example, Bottagisio et al.[18] found that PLGA screws were more effective than titanium screws in promoting bone healing and improving biomechanical properties in a rabbit model of tibial fractures. Similarly, Wang et al.[19] reported that PLGA scaffolds were more effective than titanium scaffolds. In addition, other studies have reported the potential advantages of GO as a biomaterial. Kang et al.[20] found that GO-coated titanium implants exhibited enhanced osseointegration and bone formation compared to uncoated titanium implants.

While our study adds to the growing body of evidence supporting the use of biodegradable polymers and GO in fracture fixation, there are still several limitations that should be acknowledged. First, our study was performed on rats, and the results may not necessarily apply to humans. Second, we only assessed the efficacy of GO-PLGA plates and screws in the short term (8 weeks); the long-term effects of these materials on bone healing and biomechanical properties remain unclear. Finally, although we observed improved bone healing and biomechanical properties with GO-PLGA plates and screws, the cost and availability of these materials may be a limiting factor in their widespread use in clinical practice.

Titanium plates are commonly employed in maxillofacial trauma treatment because of their favorable properties, including low density, lack of toxicity, low modulus of elasticity, and ease of shaping at room temperature.[21] However, patients undergoing mandibular fracture repair with titanium plates often experience long-term complications, such as infection, plate exposure, sensitivity to cold weather, and pain upon palpation. The use of resorbable plates for bone ossification in pediatric cases and their

impact on bone strength during the healing process is a subject of ongoing debate.

Graphene oxide-doped poly-lactic-co-glycolic acid (GO-PLGA) nanofiber absorbable plates not only significantly contribute to bone ossification, but also enhance resistance to tension and load. They offer more advantageous long-term outcomes than titanium plates when appropriate conditions are met.[22] Maxillofacial trauma is a common occurrence in clinical practice, and the implementation of GO-PLGA nanofiber absorbable plates can effectively address long-term complications associated with titanium plates, promoting superior bone healing and strength and leading to enhanced patient satisfaction.

Despite being in the experimental stage, we anticipate widespread use of GO-PLGA nanofiber absorbable plates in the future. It is important to note that GO-PLGA plates exhibit promising characteristics for biomedical applications, as evidenced by in vitro and in vivo studies that demonstrated good biocompatibility and controlled biodegradability. However, potential contradictions may arise owing to the characteristics of GO rather than the specific features of the PLGA matrix for intended biomedical applications.

While some studies have shown no particular risks associated with graphene family nanomaterials, others suggest potential health hazards. In vivo studies have emphasized factors such as lateral size, with larger GO flakes posing a higher risk of lung damage when directly injected into animal lungs. Surface characteristics and dispersibility play a crucial role in interactions with molecules, proteins, and cells, thereby influencing biocompatibility. Although detailed examinations of the interactions of graphene nanomaterials with different organs have been published, these primarily cover preliminary data for assessing their safety profiles.[23]

To the best of our knowledge, there are no commercially available bone plates integrating GO and PLGA. It is crucial to emphasize that the transition of these materials from research to commercial medical devices involves a meticulous process that includes rigorous safety assessments and compliance with regulatory standards.[24] The availability of GO-PLGA plates is contingent on ongoing research and development efforts aimed at addressing safety concerns and securing regulatory approvals.[25]

In biomedical applications such as drug delivery, GO and GO functionalized with nanoparticles or polymers are commonly used because of their cost-effectiveness and straightforward synthesis. Additionally, there is continuous emphasis on the rapid and fully scalable synthesis of graphene family materials. The costs of applying GO to PLGA bone plates are not expected to be high based on the properties of GO; however, actual costs may be influenced by factors such as production scale, materials used, manufacturing processes, and regulatory compliance.[26]

Moreover, PLGA undergoes hydrolysis in the body, breaking down into lactic and glycolic acids, which are then metabolized and eliminated. The absorption rate is influenced by factors such as the lactide-to-glycolide ratio in the PLGA polymer as well as the size and shape of the material. Commercially available Lactosorb® and RapidSorb®, both consisting of 82% lactide and 18% glycolide, are designed for midface and maxillary osteosynthesis, providing sufficient strength for 6–8 weeks and complete resorption within 12–18 months.[27]

However, there are variations in the resorbability/biodegradability of graphene-based biomaterials.[28] The biodegradation rate depends on factors such as the oxygen content, functional group nature, defects, lateral size, and number of layers.[29] Enzymes such as peroxidases have been shown to catalyze the degradation of GO. Studies have indicated that the incorporation of graphene-based materials into degradable biomaterial systems can ensure their biodegradability.[30]

In conclusion, combining graphene with degradable biomaterials such as PLGA may influence the overall degradation kinetics of composite materials.[31] Studies on GO-PLGA nanofibrous membranes in a rabbit supraspinatus tendon repair model demonstrated membrane degradation by the fourth week in both the PLGA and GO-PLGA groups, with complete degradation by the eighth week [32]. During this period, the interfacial tissues aligned regularly, and the cellularity in the GO-PLGA group decreased from 8 to 12 weeks.

Conclusion

Titanium plates are used as standard treatment in mandibular corpus fractures. However, in our study, GO-PLGA fusible plates were found to be superior to titanium plates in terms of bone stability and healing. In our study, a bone compression test was used to test bone stability, and a histological examination was performed to determine bone healing. The effect of plates on bone stability and healing can be compared with more detailed experimental studies. After these studies, we hope that GO-PLGA plates will be used in clinical studies on mandible fractures.

Ethical approval

Our study was approved by our hospital's ethics committee (number 2020-51).

Disclosure

None financial disclosure.

Funding

None.

Declaration of Competing Interest

None declared.

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