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THE EFFECT OF BIOCOMPOSITE GRAFTING MATERIAL ON CALVARIAL BONE REGENERATION (AN EXPERIMENTAL STUDY)

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ABSTRACT

INTRODUCTION: regenerating bone deficiencies produced by trauma or periodontal disorder is a major objective. The surgeon constantly seeks to collect enough bone to finish his tasks as well as to replace the amount of bone that has been lost. In oral surgery, bone grafting is a method frequently employed to make up for lost hard tissue.
AIM OF THE STUDY: comparing the bone healing rate of critical defect sizes in the calvaria rabbit with and without using a bio-composite material, both histopathologically and histomorphometrically.
MATERIALS AND METHODS: Two bone deficiencies were prepared on the Rabbits' calvaria of. The right-handed defect was the control and the left-handed defect was the study group in all of the rabbits. The control had the defect left without anything, while the study group was received biocomposite material (bone collagen, bone sulfated glycosaminoglycans sulphated glucosamine glycans (sGAG) and hydroxyapatite in natural form). At 2, 4, 6 weeks intervals, the animals were euthanized.
RESULTS: The histopathological and histomorphometric results revealed a new bone formation in both groups, the study group showed enhanced quality and quantity.
CONCLUSION: The current histological and histomorphological investigation of rabbit cranial bone deficiencies after treating with the test resorbable alloplastic graft showed an excellent biocompatibility of both the biomaterial and the distinct newly formed bone after a 6 weeks period of healing.
KEYWORDS: Bone healing, hydroxyapatite, rabbits, critical size defect, bone regeneration.
RUNNING TITLE: Bone graft in critical size defect of rabbit.

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INTRODUCTION

With few effective therapeutic strategies available, bone abnormalities brought on by infection or trauma hinder or entirely prohibit clinical healing. To control how wounds heal and allow for the regeneration of missing tissue, tissue engineering has been used (1).

In orthopaedic surgery, bone grafting is a method frequently employed to make up for lost hard tissue. This technique is frequently required to fix bone abnormalities brought on by congenital deformities, bone atrophy, tumour procedures, or traumas (2). For this, a variety of organic and artificial graft materials are employed. Autogenous grafts have long been considered as the golden rule in bone replacement because they contain osteogenic cells and osteoinductive elements that are essential for bone healing. The increase in morbidity caused by the need for a second surgical site and the potential difficulties that could occur during or after surgery are the main drawbacks of autogenous bone grafts. Resently, as a consequence, the use of different types of grafts, either alone or in conjunction with other materials, has begun to gain footing in clinical practice (3).Bio-composite material is an extremely purified bone matrix with conserved collagen and mineral constituents and natural architectonics, affinity-bound bone sGAGs of at least 1.5 mg/cm 3. It is an osteoconductive and osteoinductive porous biomaterial for filling the volume of a bone defect

or cavity. Selective binding of blood platelets to bone sGAG allows creating a chemically fixed, stable concentration of mineral components on the material, immediately triggering a cascade of reactions of bone matrix formation, without additional manipulations with the patient's blood. The material is characterized by high biocompatibility with bone tissue, low antigenicity, and being not immunogenic (4).

To increase the functional characteristics and improve its adaptation, the material should be pre-moistened with sterile saline solution for 3-5 minutes. After that, the material is placed into the bone defect after the necessary surgical procedures.

Osteomatrix [Sofikar s.r.o. (Russia)] is a biocomposite material containing bone collagen, bone sulfated glycosaminoglycans (sGAG) and hydroxyapatite in natural form. Resorption and remodeling of this material in the foci of bone tissue formation occurs within 1.5 to 3 months from the moment of implantation (5).

Since the 1970s, hydroxyapatite, the primary component of this substance, has received the most attention from researchers working in clinical settings (6). For countless years, even though HA has been applied as a non-absorbable, and as a biocompatible bone substitute, the growth of the absorbable bone substitutes has recently received considerable attention (7). Ceramic hydroxyapatite (another term for HA), is applied for the filling of bone defects. The fact that several calcium-based ceramic material including HA have been shown to assist bone ingrowth qualifies them as bioactive materials (8). Their osteoconductive qualities, which permit osteoblasts to appoint and migrate at the material surface, are related to their bioactivity (9). It is well known that HA can adhere to bone directly (10). In dentistry and maxillofacial surgery, HA has been utilised to assist alveolar bone regeneration with sufficient clinical success rates, either alone or in combination with an auto/allo/xenograft (11). HA comes in a wide range of forms (powders, porous blocks, or beads).

The present research was designed to evaluate the osteomatrix effects on healing a critical size defect in calvarial bone of rabbit.

In this study, the **null hypothesis** is that there may not be any significant differences between either the presence or absence of bio-composite material in acceleration of bone healing in the calvarial critical size bone defect in rabbit.

MATERIALS AND METHODS

Study Design

The study was an experimental animal study.

Study sample and setting

This study included eighteen male Egyptian Rabbits with a mean age of one year and weighing 3.5-4 Kg. Animals were obtained from the animal house of Medical Research Institute, Alexandria University. The rabbits were kept under the same environmental conditions in the experimental animal house. The histological procedures were performed in the laboratory of the Oral Biology Department at the Faculty of Dentistry, Alexandria University. The present study was approved by the ethical committee of Faculty of Dentistry, Alexandria University.

Randomization

Coin toss was used to determine which side of the rabbit was used as test and which was control.

Animal housing (12)

The rabbits were kept under normal ventilated laboratory conditions of temperature (22-25°C). Rabbits were housed in cages fed by standardized suitable food and tap water. The standard diet regimen was replenished daily throughout the experimental period.

Eligibility

The selected Rabbits were according to:

Inclusion criteria

1. Average weight 3.5-4 Kg.
2. Healthy mature rabbits.
3. One year age
4. No systemic disease.

Exclusion criteria

1. Rabbits that are over weighted or underweighted.
2. Presence of any illness or wounds.
3. Rabbits younger than one year age.

Sample Size (S. S. Calculation)

Based on their results, adopting a power of 80% to detect a standardized effect size (d) in mean percentage of newly formed bone after 6 weeks (primary outcome) =1.870 (large-sized standardized effect size), and level of significance 95% (α=0.05), the minimum required sample size was found to be 6 defects per group (20), (number of groups=2) (Number of scarification intervals=3)

Total sample size = Number per group × Number of groups × Number of scarification intervals. 6×3×2=**36 defects**.

Any sample withdraws from the study was replaced to maintain the sample size (13).

The sample size was calculated using GPower version 3.1.9.2 (14).

Methods

Preparation

All of the operative procedure were performed under general anesthesia. The rabbits were anesthesized by intramuscular injection of (0.15-0.20mg)/Kg ketamine plus (1-2mg)/Kg lidocaine (15, 16).

The surgical area in the calvarial of all the rabbits were shaved before any procedure and the skin was rinsed and scrubbed with 2% povidone iodine to avoid contamination.

Surgical procedure

After anesthesia, a 4 cm incision was made by surgical blade number 15, the surgical site was exposed with a sagittal midline incision through the skin and the periosteum at the midline of the

calvaria and a full-thickness flap elevation (17). For each rabbit: two defects were created the right (control) and left (study) using 8 mm diameter sterile surgical bur (Trephine bur) with profuse saline irrigation to protect bone from heat generation. The defect. The control defect was left empty while the study defect received biocomposite material osteomatrix crumb SOFIKAR company (bone collagen, bone sulfated glycosaminoglycans (sGAG) and hydroxyapatite in natural form). The surgical area was sutured by 000 non absorbable braided suture. All rabbits received the same course of antibiotics amoxicillin 1gm/Kg body weight every eight hours for five days.

Animal euthanasia (18)

The rabbits were euthanized with an overdose of ketamine at each of the experimental periods 2, 4 & 6 weeks postoperatively. The calvaria specimens were obtained. The defect area was dissected out and processed for light microscopic examination and histomorphometric analysis.

Disposal of the animals

Rabbits were disposed by burning by special authorities.

Histological examination

Specimens were fixed in 10% neutral buffered formalin, washed, and then decalcified. After washing, the specimens were dehydrated in ascending grades of alcohol, cleared in xylene, and infiltrated and embedded in paraffin wax. Serial sections of 5 µm thick were cut from the paraffin blocks using a rotary microtome and stained with hematoxylin and eosin. The stained sections were assessed for the formation of new calcified bone by evaluation under an optical microscope (11).

Histomorphometric analysis

Histomorphometric evaluation of the newly formed bone was performed using ImageJ 1.46r software to compare the percentage of the surface area of the newly formed bone in different groups (19).

Ethical Considerations

The study was performed after gaining the approval of the Research Ethics Committee, Faculty of Dentistry, Alexandria University.

According to American Animal Welfare Act (AWA) provision, there are new minimum standards of care for handling, housing, feeding, water, sanitation, and ventilation. Besides, the researches facilities must have procedures that minimize pain and stress to the animals (20).

Animal safety

The animals were housed in specially designed wire mesh bottom cages and received water and diet throughout the experimental period. The animals were maintained under controlled cycle of temperature and humidity. During experimental duration they were housed in cages that permit suitable ventilation. The research had approaches that achieve no or least pain or stress to the rabbits.

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric.

The comparison between two independent groups with quantitative data and parametric distribution was done by using **Independent t-test**.

The comparison between more than two paired groups with quantitative data and parametric distribution were done by using **Repeated Measure ANOVA**.

The comparison between two paired groups with quantitative data and parametric distribution were done by using **Paired t- test**.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

- $P > 0.05$ = non significant (NS)
- $P < 0.05$ = significant (S)
- $P < 0.001$ = highly significant (HS).

RESULTS

As illustrated in **Fig (1). Table (1)** shows that there was highly statistically significant difference found between Control group and Study group regarding Bone Formation in 2 weeks.

As illustrated in **Fig (1). Table (1)** shows that there was highly statistically significant difference found between Control group and Study group regarding Bone Formation in 4 weeks.

As illustrated in **Fig (1). Table (1)** shows that there was highly statistically significant difference found between Control group and Study group regarding Bone Formation in 6 weeks.

As illustrated in, **Fig (2). Table (2)** shows that there was highly statistically significant difference found between bone formation in 2 weeks, bone formation in 4 weeks and bone formation in 6 weeks, while there was statistically significant difference found between bone formation in 2 weeks and bone formation in 4 weeks, and there was highly statistically significant difference found between bone formation in 2 weeks and bone formation in 4 weeks, and there was highly statistically significant difference found between bone formation in 4 weeks and bone formation in 6 weeks

Table (1): Comparison between Control group (no. =6) and Study group (no. =6) regarding Bone Formation in 2, 4 and 6 weeks

	Control group	Study group	Test value•	P-value	Sig.
	No.= 6	No.= 6			
2 Week					
Mean ± SD	25.08 ± 3.66	32.67 ± 5.69	-2.750	0.002	HS
Range	20.09 – 30.87	24.00 – 41.44			
4 Week					
Mean ± SD	34.22 ± 5.08	44.02 ± 5.02	-3.361	0.007	HS
Range	24.65 – 39.83	37.52 – 51.41			
6 Week					
Mean ± SD	47.07 ± 2.72	58.82 ± 5.73	-4.535	0.001	HS
Range	42.62 – 49.91	51.58 – 65.39			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
•: Independent t-test

Table (2): Comparison between Bone Formation in 2 week, Bone Formation in 4 week and Bone Formation in 6 week in Control and study groups

	2 week	4 week	6 week	Test value*	P-value	Sig.
Control group						
Mean ± SD	25.08 ± 3.66	34.22 ± 5.08	47.07 ± 2.72	1683.903	0.000	HS
Range	20.09 – 30.87	24.65 – 39.83	42.62 – 49.91			
	Post hoc analysis€			–	–	–
	P1	**P2**	**P3**	–	–	–
	0.005	0.000	0.008	–	–	–
Study group						
Mean ± SD	32.67 ± 5.69	44.02 ± 5.02	58.82 ± 5.73	1792.719	0.000	HS
Range	24.00 – 41.44	37.52 – 51.41	51.58 – 65.39			
	Post hoc analysis€			–	–	–
	P1	**P2**	**P3**	–	–	–
	0.020	0.001	0.003	–	–	–

P-value >0.05: Non significant(NS); P-value <0.05: Significant(S); P-value< 0.01: highly significant(HS)
*: Repeated Measure ANOVA; €: Paired t-test

2 Weeks
Histological evaluation of the H&E stained sections in the control group after 2 weeks showed dense fibrous tissue bundler filling the bone defect and encapsulating remnants of old bone embedded accidently within the defect during its preparation. Inflammatory cells were also revealed. The necrotic old bone showed empty osteocytes lacunae.
The margins of the defect were irregular indicating active bone resorption by osteoclasts.
The study group revealed the arrangement of osteoblasts on the peripheries of the bone defect. The deposition of osteoid was also revealed in addition to the bone inclusions surrounded by fibrous bondles.

4 Weeks
After 4 weeks specimens of the control group showed an empty bone defect without any signs of bone formation dense fat cells were observed within the marrow cavities. Condensation of inflammatory cells were also revealed.
The study group defects showed the formation of new spondy bone surrounding the graft material. Continuous layers of plump osteoblasts were revealed indicating active bone formation. Network of collagen fibers were observed in addition to dense fibrous tissue bundles at the center.

6 Weeks
Observation of the H&E stained sections after 6 weeks in the control group revealed the formation of scattered thin trabeculae of cancellous bone lined by resting cells surrounding the fatty marrow tissue. Reduced cellularity and vascularity was also noted.
However, the study group showed a meshwork of mature bony trabeculae filling the bone defect formed around the allograft particles.
The marrow cavities were lined by flat endosteal cells and comprised areas of fat cells.
Resting and reversal line were observed indicating bone maturation and remodeling segments of deeply stained compact bone osteons were also revealed fused to the surrounding cancellous bone.

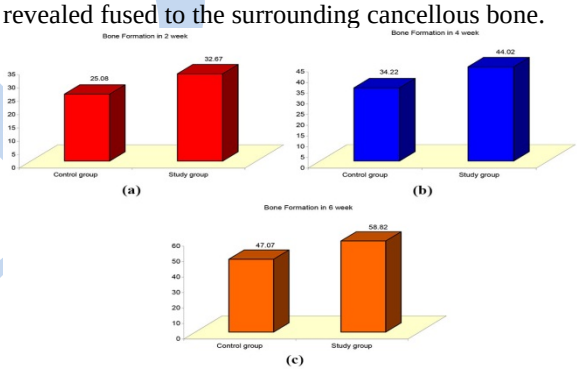

Figure (1): The difference between (Control group and Study group) regarding Bone Formation in 2 weeks, 4 weeks and 6 weeks.

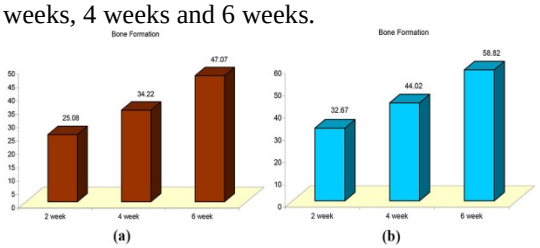

Figure (2): The difference between Bone Formation in 2 weeks, Bone Formation in 4 weeks and Bone Formation in 6 weeks in Control and study group.

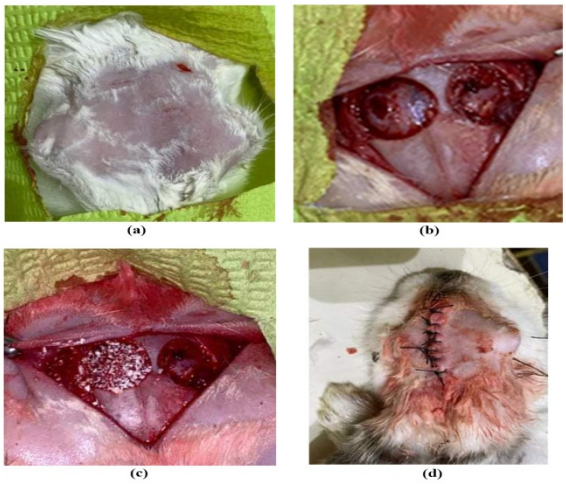

Figure (3): Surgical procedure a) shaving, b) flap reflection & bilateral defects formed, c) right defect with graft inside, d) suturing of the wound

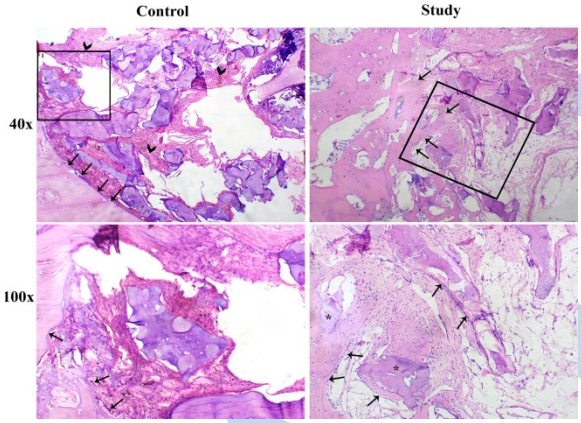

Figure (4): Control (40x) Light micrograph (LM) of the control group after 2 weeks showing the margins of the critical size defect (arrows) filled with remnants of the old bone embedded during the surgical procedures surrounded by dense fibrous tissue (arrow heads). Few inflammatory cells also noted (H&E, × 40). **Control (100x)** LM showing higher magnification of the previous micrograph inset showing the necrotic bony fragments with empty osteocytes lacumaen Note the irregularities on the defect borders indicating the start of the resorption (H&E × 100). **Study (40x)** LM of the study group after 2 weeks showing the alignment of bone forming cells (arrows) on the surface of the bone defect also filled with some bony fragments encapsulated by fibrous bundles. (H&E, × 40). **Study (100x)** Higher magnification of the previous micrograph inset showing the arrangement of plump osteoblasts on the peripheries of the bone defect and surrounding the graph material (asterisks) and the deposition of organic matrix with trapped osteocytes cells. (H&E × 100)

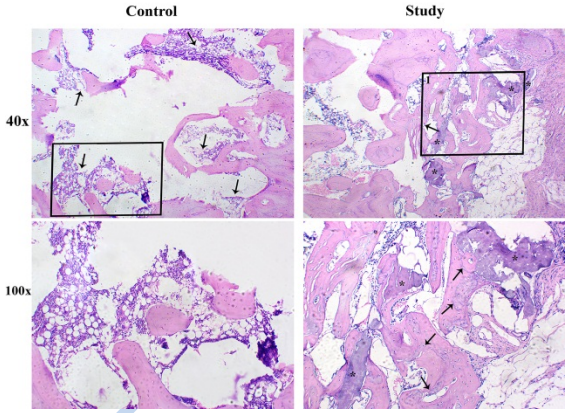

Figure (5): Control (40x) LM of the control group after 4 weeks showing empty bone defect without any sign of bone formation. Note the fatty tissue infiltration. (H&E, × 40). **Control (100x)** Higher magnification of the previous micrograph inset showing the fat cells surrounded by condensed inflammatory cells. Note the absence of surface osteoblasts and compromised vascularity. (H&E × 100). **Study (40x)** LM of the study group after 4 weeks showing the formation of bundle of new bone surrounding the graft particles (asterisk). Note the deposition of collagen fibers to form bony trabeculae. Continuous layer of osteoblasts indicating active bone formation. Thick bundles of fibrous tissue were also observed at the center of the bone defect. (H&E, × 40) **Study (100x)** Higher magnification of the previous micrograph inset showing the newly formed bone trabeculae surrounding the allograft. Well vascularized marrow tissue was noted in addition to resting and reversal lines indicating bone maturation. (H&E × 100)

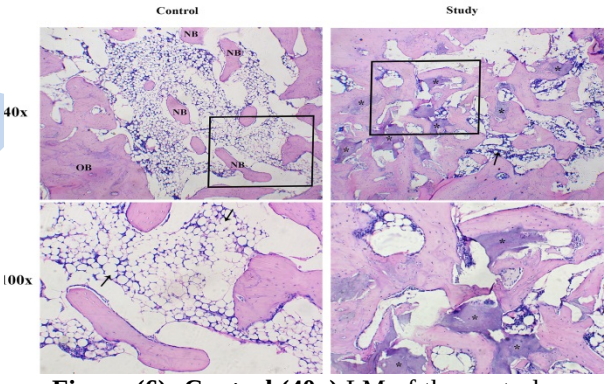

Figure (6): Control (40x) LM of the control group after 6 weeks showing thin scattered trabeculae of cancellous bone surrounded by dense fatty tissue infiltration within the marrow cavities. (H&E, × 40). **Control (100x)** Higher magnification of the previous micrograph inset showing thin dispersed segments of cancellous bone lined by resting bone lining cells and surrounded by aggregations of fat cells. Note: reduced cellularity and vascularity of the mesenchyme within the bone marrow (H&E × 100). **Study (40x)** LM of the study group after 6 weeks showing the bone defect filled by a network

of cancellous bone trabeculae surrounding the graft material (asterisk). Note few areas of fat cell, infiltrating the bone marrow (H&E, × 40). **Study (100x)** Higher magnification of the previous micrograph inset showing the mature trabeculae of spongy bone surrounding the allograft particles. Note the marrow cavities lined by flat endosteal cells with scattered groups of fat cells. (H&E × 100)

DISCUSSION

The absence of the bone tissues in a specific body constituent which might develop as a trauma consequence, infection, inflammation, congenital, or traumatic incidents is referred to as a bone deficiency. Also, it may occasionally occur as an outcome of persisting clincal conditions or surgical treatments. Bone repair by the surgical insertion of materials is frequently necessary to stimulate bone regeneration when the regeneration process in bone loss is slowed or even interrupted (21).

Recently, Because of their superior bone conduction, inducibility, and osteo-genesis, auto-graft material has been the principal source of material utilized for bone restoration. In practical practise, an autologous bone transplant is still the go-to therapy for bone repair. The restricted availability of donor bone and harvesting-related problems, however, limit the practical use of this approach (22).

As a result, various bone substitutes are essential for effective bone restorations as alternate graft materials. Being osteoinductive, osteoconductive, and bioresorbable are all qualities that a perfect bone replacement should possess. Additionally, it should not be susceptible to immunological rejection and be thermally nonconductive (23).

Our current work revealed an extremely statistically noteworthy difference between both the control and the study group in regards to the formation of bone in 2 weeks, 4 weeks, and 6 weeks when comparing the two groups based on bone formation in 2, 4, and 6.

Ossification—the process of creating new bones—was not occurring in any of the groups during the 2-week observation. by the 4-week' end, we observed that each group had initiated an ossification process. With only a tiny amount of osseous fragment, the deficiencies in the blank group were filled with fibrinous connective tissue. Both the research and control groups mostly included fibrinous connective tissue, fatty tissues, and branching, prosperous trabecular bone. At this point, tissue remodeling was under way, and bone marrow and adipose tissue infiltration helped the new bone anneal to the lamellar bone. Original structure was not recovered in the blank group six weeks after implantation (only a small amount of new bone formation).

This study employed a rabbit model because, while being quicker, a rabbit's bone-repair process is physiologically similar to that of a person. Additionally, using cranial bone for grafting offers several notable advantages, including a greater percentage of surviving bone transplant and a relatively quick postoperative recovery period (24).

The histological results of the current investigation showed a clear boost in new bone production and a considerable reduction in concentration of inflammatory cells of the osteomatrix filled defects. Unfortunately, little literature has used osteomatrix collagen bone graft in calvarial bone defects.

These outcomes are consistent with those observed by Xiaoxia et al., who also noted that the tested PAA/HA/CS composites had high heat resistance. Rabbit muscle implants made of PAA/HA/CS composite were examined histologically, and the results showed that the composite had good biocompatibility. The composites were directly associated with the hosting tissue without any encapsulating connective layer when implanted in the rabbits' muscles, showing high biocompatibility. The material's mechanical strength, biocompatibility, osteo-conductivity, and processability made it a great option for usage as a substituent for the cranial bones (25).

The lowest diameter osseous defect that does not mend on its own is termed to as a critical-size defect (CSD). In calvaria rat, a full defect thickness of (8mm) diameters has been presented as a CSD. Bone substitutes that are commercially obtainable didn't exhibit osteo-conductive qualities or impair the production of newly bones in rat calvarial lesions. According to a number of previous experimental investigations, It has been reported that the non-CSD might also be substantial (26).

According to other research, rat calvaria with flaws of 5 or 6 mm in diameter also meet the criteria for a CSD (27, 28). A 5-mm-diameter calvarial defect, according to Park et al., is not a CSD in rats since, at 8 weeks, unfilled lesions had a high degree of new bone growth. In this investigation, a CSD diameter of (8-mm) was designed in the calvaria rat for a cautious inspecting that depended on these literature reviews (29).

In contrast to genuine bone, which contains 2.3–8 percent carbonate per gramme, hydroxyapatite (HAp) has a poor solubility due to its low carbonate component and a sluggish rate of absorption. These drawbacks are improved when the functional structures of HAp, OH or PO4, are switched to CO3 (30).

Ceramics made of hydroxyapatite lack osteogenecity but have high biocompatibility and osteoinductivity. Numerous HAp products appear to have varying osteoinductivities, with porous architectures, the degree of pore interconnection, and linked pore size being some of the contributing factors (31).

Osteocytes can differentiate when they receive enough nutrients through angiogenesis and the linked pore size is more than 100 m. However, the ideal size was said to be 300 m in the literature, and

when linked pores are too large, bone formation might be impeded. Pore size of the osteomatrix employed in this investigation is 300 m (32).

For many years, anorganic bone made from cow bone has produced good results in a variety of human and animal trials; nonetheless, the newly created bone has shown significant differences around the range (5-42%) (32).

According to **Jensen et al.,** at the mandibular angle of minipigs, new bone development took up 4% (2 weeks), 26% (4 weeks), and 42% (8 weeks). Although the percentage was lesser than of the autogenous bone or tricalcium phosphate (TCP), the outcome was nevertheless viewed favourably (32).

Human samples that were taken 11 years following augmentation of the sinus floor with de-proteinized bovine and autogenous bone were subjected to histological and histomorphometrical investigations by **Mordenfeld et al. (33).** They revealed that the xeno-grafted particles were well-integrated into the lamellar bone and did not undergo any notable changes in particle size. Another crucial point is that there are still serious worries that people who get bone transplants from cattle run the danger of contracting prions.

Kim et al. claim that it is challenging to screen for prions inside the animal genome. Infected patients also have a lengthy latency period (from one year to more than 50 years) before symptoms of bovine spongiform encephalopathy develop. These elements offer a framework for the consideration of potential long-term dangers associated with the widely utilised xenografts in dentistry (34).

In surgically made bone lesions on the iliac crest of Beagle dogs, **Podaropoulos et al.** (35) observed 21.62 percent of residual -TCP/CS four months following implantation of the material, although a prior animal research had shown total biodegradation of the -TCP/CS graft after 36 weeks after implantation (29).

The amount of regenerated bone tissue may be significantly impacted by the use of grafting materials to correct bone defects, and the presence of graft particles may change the microarchitecture of the newly created tissue. The shape of the newly produced tissue and the pace of bone resorption appear to be influenced by the grafting material's capacity to aid bone regeneration. These variations may have an impact on the overall quality of the developing bone (36).

Clinical relevance and implications

In recent years, the autologous tooth-derived graft materialinistration of drugs acting on vascularization and bone metabolism and the evidence of chronic infections could determine an increased risk of implant failure.

The need to develop new, more efficient materials and techniques for functional and aesthetic rehabilitations remains a priority for researchers in the dental field. A biomaterial that is used as a bone substitute should possess certain qualitative criteria: biocompatibility, which represents the capability of providing osseointegration without causing inflammatory reactions, osteoconductivity, the natural properties that allow cell activity, reproduction and amplification, and at last osteoinductive properties, or to be capable of triggering the bio-chemical and modulating processes, so stem cells can differentiate into osteoblasts, osteoclasts and osteocytes and induce osteogenesis, which is related to the formation of a new bone matrix. Moreover, osteoinductive biomaterials should be able to recruit progenitor cells (MCS) to the grafted site, induce the formation of osteoblasts by differentiating progenitor cells (MCS) in mature cells and eventually regenerate ectopic bone where there is no extraskeletal structure (37).

Bone tissue engineering (BTE) is a newly developed alternative approach that introduces an exogenous scaffold to encourage cells to grow and proliferate by providing regulatory growth factors. These implantable scaffolds are required to match the properties of the original tissues, such as osteoinductivity, biocompatibility, osteoconductivity, and suitable mechanical strength. Various artificial implantable scaffolds, including metal, bioceramic, biopolymer, and composite implants, are manufactured for bone tissue regeneration or BTE applications. Although bone regeneration shows excellent promise, BTE still has some fatal problems, such as high cost, the potential tumorigenic risk of growth factors, and the inability to develop a natural combination with the surrounding normal tissue. Moreover, implant fractures caused by inflammation, loosening, and osteolysis remain as challenges to be addressed (38).

Future perspectives

Bone grafts have certain shortcomings, especially which have growth factors. Firstly, protein molecules in the body undergo rapid biodegradation from exudation and proteolytic enzymes limiting their osteoinductive action. Second, the therapeutic protein acts short term, and it is difficult for controlled release. In addition, newer technologies such as growth factors using recombinant growth factors also have limitations in using such biomaterials in surgery for early release in wound healing. Another emerging technique for the delivery of growth factors is gene therapy, where genetic material is transferred into the genome to produce specific action through a functional protein, such as BMP. The biodegradable scaffolds are developed to maintain space to promote vascular ingrowth, and cell adhesion (39).

Various techniques can be used to study the bone structures, such as cell staining, infrared absorption spectroscopy, and CBCT. In addition, it is important to consider technological evolution to reduce the damage and side effects of necessary diagnostic tests. This can be done by specifying the difference between radiation-free and nonradiation in evaluating the effects. The use of ultrasound in dentistry can represent a radiation-free alternative to the other most used exams (40).

Similarly, digital technologies are at the forefront for integrating 3D and 4D printing with other technologies that can be applied in implant dentistry. A CBCT of the jaw can produce virtual planning of the reconstruction using software and can produce a 3D model of the jaw for reconstruction. Furthermore, allogeneic graft and xenograft block bone grafts may be milled to make custom-fit. In addition, custom-made resorbable scaffolds or custom titanium meshes can be fabricated containing growth factor-enhanced grafts routinely using a 3D printer (40).

CONCLUSIONS

This study looked at new bone development in critical-sized bone defects in rabbit calvaria. Similar to the impact of new bone creation without it, HAp linked bone tissue and offered useful room for future bone ingrowth.

The test resorbable alloplastic graft repaired rabbit cranial bone lesions, and the current histological and histomorphological analysis of those defects showed outstanding biocompatibility of the biomaterial and pronounced new bone production after a healing time of six weeks.

Regarding to the fact that our work didn't consider time points before 12 weeks, the current study has several limitations. Prior to 12 weeks, it may be possible to find more variations between materials and the earliest cellular processes that control healing by examining the in vivo response.

Additionally, we did not look at the whole range of collagen-degrading enzymes that may affect the inflammatory response through the healing process. It is envisaged that further advancements in biomaterial synthesis and processing, as well as our capacity to distinguish them in animal models to give insight into bone repair, will enhance patient results.

Conflict of interest

The authors declare that they have no conflict of interest.

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