EVALUATION OF USING MINERALIZED PLASMATIC MATRIX IN OSSEOUS REGENERATION (EXPERIMENTAL AND HISTOMORPHOMETRIC STUDY ON RABBITS)

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ABSTRACT

INTRODUCTION: The use of platelet concentrates is recent and its efficiency remains controversial. The mineralized plasmatic matrix (MPM), is the latest innovation of platelet concentrates (i.e. PRP and PRF). A new concept of fabricating growth factors-enriched bone graft matrix has been introduced since 2010. It utilizes altering the centrifugation speed and time to produce much larger, denser and richer fibrin matrix containing growth factors, known as Sticky bone. MPM combines the best of both worlds; its mineral phase qualifies it as an osteoconductive scaffold for bone formation while its platelet concentrate phase grants it an osteoinductive property by the slow release of growth factors.

OBJECTIVES: To evaluate the effectiveness of using MPM, compared to the use of bone graft alone, to assess the exact effect of each in enhancing the osteogenic differentiation and bone formation.

MATERIALS AND METHODS: Ten healthy adult white Albino New Zealand rabbits, after creating two osseous defects in the right femur in each. The defects (upper and lower) were divided in to two groups according to the material used for filling:

- Group A (Control): in the upper defect, bone graft only was added in the lateral side of the right femur.
- Group B (Study): in the lower defect, MPM was added in the lateral side of the right femur.

After 2 & 8 weeks respectively postoperatively, sacrifice of the rabbits was done.

RESULTS: Data collected from histological results revealed that MPM enhanced bone formation where increased amount of new bone formation was observed in the study group in relation to the control group. At the end of the experimental period, the defect area was almost filled with mature bone which occupied a greater surface area in the study group. Histomorphometric analysis revealed that there was increase in the mean percentage of bone surface area in the study group in comparison to the control group. This increase was statistically significant in the 8 weeks period.

CONCLUSIONS: MPM proved its efficiency in enhancement of osseous regeneration.

KEYWORDS: Platelet rich fibrin (PRF), MPM (Mineralized plasmatic matrix), Platelet rich plasma (PRP), bone graft, osseous regeneration.

INTRODUCTION

The repair and regeneration of bone is a major issue in the Oral and Maxillofacial field and for the whole human body in general. Bone loss is caused by many diseases (congenital or degenerative), traumas and surgical procedures; it is a problem for functionality and is having an ever-increasing social impact (1).

Bone grafting is often necessary to provide support, fill voids and enhance biologic repair of skeletal defects (2). It includes autografts, allografts, xenografts and alloplastic materials (3,4).

Autogenous bone is an ideal bone graft having osteogenic, osteoconductive and osteoinductive potential. Donor site morbidity such as neurovascular injury, infection, haematoma formation and chronic pain are well known complications of autogenous bone graft as well as it is limited in quantity (5). Therefore; efforts have focused on finding ideal materials that could be used as substitutes for autografts (6). The selection of the most appropriate scaffolds is an important step towards the regeneration of hard tissue (7).

Platelet-derived Growth Factors are biologically active peptides that enhance tissue repair mechanisms, cell proliferation and regulate angiogenesis (8).

Platelets are easily collected from the blood stream and are concentrated in a small volume of plasma known as platelet-rich plasma (PRP) (9). It is the first form of autologous platelet concentrate to replace commercial fibrin glue. The preparation of PRP involves the isolation of PRP after which gel formation is accelerated using calcium chloride and bovine thrombin. It has been discovered that the use of bovine thrombin may be associated with the development of antibodies to the factors V, XI and thrombin, resulting in the risk of life coagulopathies. Bovine thrombin preparations have shown to contain factor V, which could result in the stimulation of the immune system when challenged with a foreign protein (10).

Platelet rich fibrin (PRF) was first described by Choukroun et al. (11). This second-generation platelet...
concentrate eliminated the risks associated with the use of bovine thrombin (12). The natural concentrate of PRF is produced without any anticoagulants or gelifying agents (13). It is an autologous fibrin matrix containing many kinds of growth factors and cytokines (14). Growth factors are known to promote cell proliferation, cell differentiation, cell motility, and matrix synthesis by binding to specific cell-surface receptors (15). It is generally accepted that growth factors have an essential role in the healing process and tissue formation (16,17).

Platelet rich fibrin has several advantages over traditionally prepared platelet-rich plasma including ease of preparation and lack of biochemical handling of blood which makes this preparation strictly autologous (18). It can be used in conjunction with bone grafts, which offers several advantages including promoting wound healing, bone growth and maturation, graft stabilization, wound sealing and hemostasis, and improving the handling properties of graft materials (19).

The latest innovation of platelets concentrates (i.e PRP and PRF) is the mineralized plasmatic matrix. Therefore, it has been suggested that upon further optimization, MPM may serve as a third generation of platelet concentrate with potential application in various fields. The MPM is a modification of and PRF initially presented by Perisse (20) in 2012 and modified by ElMoheb (21) in 2014. The advantage of the MPM is the integration of bone grafts particles inside the fibrin network that is not present in old autologous growth factors membranes in PRF or PRP. In fact, the bone grafting materials are prepared and mixed with the autologous growth factors to produce the MPM or what is called Sticky bone. This offers the MPM the positional stability by stabilizing the bone particles, preserving its shape with subsequent “in situ” immobilization of the component of ridge preservation materials (22).

In this study, we introduced an easy technique utilizing (MPM:mineralized plasmatic matrix) for reconstruction of bony defects and evaluation ofits effectiveness in osseous regeneration.

MATERIALS AND METHODS

Methods
The experimental procedure was held in the animal house at Faculty of Agriculture, Alexandria University. Animals were kept under safe conditions during the time of the study. Rabbits were kept under the same nutritional and environmental conditions throughout the whole period of the study. Each animal received a dose of antibiotics in the form of Amoxicillin (Epicoill, EPICO, Cairo, Egypt) 25 mg/kg body weight by intra-muscular injection just before the operation.

Operative phase
Preparation of MPM
- 5 CC of rabbits venous blood was taken from the marginal ear vein, and the blood was added to the non-coated plan vacutainers. The blood in the test tubes was centrifuged at 2400-2700rpm for 2-4 minutes (20,21). The non-coated tube showed 2 different layers:
  - The upper layer showed a liquid yellow plasma while the lower layer showed red blood cells layer which was discarded (Figure 1a).
  - The yellow part was then collected using a syringe, and added to cup that contains the bone graft material. (Figure 1b)

The whole thing was mixed for few minutes to polymerize in order to obtain the MPM (sticky bone), which is yellowish in color. (Figure 2a)

Figure 1: (A) Showing liquid yellow plasma layer on the top of the red blood cell layer after test tube centrifugation.
(B) Showing two cups, the right one contains MPM while the left one contain bone graft alone.

Anaesthesia
All operating procedures were performed under general anaesthesia. Each animal was generally anaesthetized via intramuscular injection of Xylazine HCL 2% (Xylaine) (Bayer Inc, Toronto,ON,Canada), the dose of which was calculated on basis of 2mg/kg body weight and intramuscular injection of ketamine HCL 10% (Ketamine) (Alfas an Inc.,Utrecht,Holland) in a dose of 3 mg/kg body weight.

Surgical operation
The femur area wasshaved and sterilized with bovidone Iodine (Betadine) (Nile Company, Cairo, Egypt). About 5cm incision was done. Once the skin was incised, the fascia was cut with a pair of scissors until the bone was reached. The periosteum was reflected with a periosteal elevator. Two surgically created bone defects of 5mm diameter and 5mm depth, and 5cm apart from each other were prepared in the right side by the aid of trephine bur (Tut Company, Cairo, Egypt).

The defect was filled with different materials according to the study group (Figure 2b):

Methods

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

The study that was conducted on 10 healthy adult white New Zealand rabbits (age 2-2.5 months and weighing 4-5 kgs approximately), the defects were divided in to two groups according to the material used for filling:

Group A (Control): in the upper defect, Bone graft only was added in the lateral side of the right femur.

Group B (Study): in the lower defect, MPM was added in the lateral side of the right femur.

B) Bone graft: Hypro-Oss®

Hypro-Oss® (Hypros.r.o, Germany,Europe) is a natural bovine bone substitution material for permanent filling and reconstruction of bone defects. Each granule consists of 30% Atelo-Collagen Type I and 70% hydroxyapatite. Hypro-Oss® is produced by using patented atelopeptidation and lyophilization processes.

Hypro-Oss® is a sterile medical device class III, ISO and CE-certified.
Animal Sacrification

Rabbit's were sacrificed at (2 - 8 weeks), (5 rabbits each group), respectively postoperatively. Then they also received a dose of Amoxicillin (amoxil), (GSK, Cairo, Egypt) 250 mg IM for 3 days postoperatively. The mean bone surface area of the three rectangles was calculated for each section. The mean of the values from the three sections was obtained and its percentage to the total area selected was calculated. The same procedure was repeated for each of the five animals in each group. The collected data of the percentage of bone surface area were statistically analyzed.

Statistical analysis of the data

Measurements for the percentage of bone surface area were collected from the images. Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) (26). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level, (P ≤ 0.05).

The used tests were

1- Student t-test

For normally distributed quantitative variables, to compare between two studied groups.

2- Mann Whitney test

For abnormally distributed quantitative variables, to compare between two studied groups.

RESULTS

Clinical observations

All rabbits survived through the entire study period. No significant complications or clinical signs of infection or wound dehiscence that would impair osseous regeneration throughout the whole follow up period.
Histological results
H&E stain was used to distinguish between immature woven bone and mature lamellar bone.

Light microscopic examination of the 2 groups to observe different levels of bone formation as well as the collagen content of the newly formed bone.

Two weeks interval
Light microscope examination of specimens taken from the defect site of group A (control) revealed limited amount of new bone formation extending from the margins of the defect. This newly formed bone consists of spicules containing haphazardly arranged osteocytes. The remaining part of defect contains fibrous connective tissue and remnants of the graft material. Cartilage like tissue could be also seen (Figure 3).

On the other hand, in group B (study), more numerous bony spicules were seen originating from all around the periphery of the bone defect. This newly formed bone contained numerous, large, irregularly arranged osteocytes. Osteoblasts cells were seen lining the endosteal surface of the bony spicules (Figure 4).

Eight weeks interval
Histological examination of specimens obtained from group A (control) revealed more amount of bone formation in relation to the 2 weeks group. The bone appeared denser and consisted of small newly formed osteons surrounding some remnants of the graft material. Some areas of immature bone which contained numerous irregularly arranged osteocytes were seen in between the adult bone. Complete osteointegration between the old and new bone was seen (Figure 5).

In group B (study), similar observations were noted like the control group but more amount of bone were seen filling most of the defect site. This new bone mass consisted mostly of compact bone enclosing small areas of immature bone (Figure 6).
Histomorphometric results to evaluate bone density

Table 1 showed comparison between the two studied period groups (2,8 weeks) according to the mean percentage of bone surface area in the control group (Bone graft alone). The mean percentage of bone surface area was 40.00 % after 2 weeks & 66.80 % after 8 weeks, and that increase was statistically significant as p value was p1=0.001.

Table 1 showed comparison between the two studied period groups (2,8 weeks) according to the mean percentage of bone surface area in the study group (MPM). The mean percentage of bone surface area was 47.0% after 2 weeks & 74.80% after 8 weeks, and that increase was statistically significant as p value was p2<0.001.

Table (1): Comparison between the two studied periods according to mean bone percentage results in control and study groups (n=5).

<table>
<thead>
<tr>
<th>Mean bone percentage results in control groups</th>
<th>2 weeks</th>
<th>8 weeks</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>37.0 – 42.0</td>
<td>62.0 – 70.0</td>
<td>127.59*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>40.00 ± 1.87</td>
<td>66.80 ± 3.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>67</td>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. bet. periods</td>
<td>P1=0.001*</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean bone percentage results in study groups</th>
<th>2 weeks</th>
<th>8 weeks</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. – Max.</td>
<td>42.0 – 50.0</td>
<td>71.0 – 78.0</td>
<td>77.943*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>47.0 ± 3.16</td>
<td>74.80 ± 3.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>75</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sig. bet. periods</td>
<td>P2&lt;0.001*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

t: Student t-test
p: p value for comparing between 2 weeks and 8 weeks in control groups.
p2: p value for comparing between 2 weeks and 8 weeks in study groups
*: Statistically significant at p ≤ 0.05

Table 2 showed comparison between the control and the 2 study groups according to the mean percentage of bone surface area. There was a statistically significant difference between them at the 2 periods (2&8 weeks) where the p value was (0.001), respectively.

Table 3 showed comparison between the 2 study groups according to the change in the mean percentage of bone surface area at each study period.

There was no statistically significant difference between them as p value was (p=0.008).

Table (2): Comparison between the two studied groups according to mean bone percentage results.

<table>
<thead>
<tr>
<th>Mean bone percentage results</th>
<th>Control (n=5)</th>
<th>Study (n=5)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>37.0 – 42.0</td>
<td>42.0 – 50.0</td>
<td>4.260*</td>
<td>0.003*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>40.00 ± 1.87</td>
<td>± 14.0 ± 3.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>60</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>62.0 – 70.0</td>
<td>71.0 – 78.0</td>
<td>3.961*</td>
<td>0.004*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>66.80 ± 3.11</td>
<td>± 74.80 ± 3.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>67</td>
<td>75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

t: Student t-test
p: p value for comparing between the two groups
*: Statistically significant at p ≤ 0.05

Table (3): Comparison between the two studied groups according to change of mean bone percentage results.

<table>
<thead>
<tr>
<th>Change of mean bone percentage results</th>
<th>Control (n=5)</th>
<th>Study (n=5)</th>
<th>U</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 – 8 Weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. – Max.</td>
<td>22.0 – 25.0</td>
<td>23.0 – 33.0</td>
<td>1.0</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>23.40 ± 1.14</td>
<td>± 27.80 ± 3.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>23</td>
<td>26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

U: Mann Whitney test
p: p value for comparing between the two groups

DISCUSSION

The hypothesis of this study was that the use of concentrated growth factors combined with a bone graft material could yield better results in terms of quantity and quality of osseous regeneration compared to the use of bone graft alone.

Limited studies are reported in the literature concerning the MPM. However, the effect of growth factors on bone healing and osseointegration has been extensively investigated. Lee et al. (27), in an animal study reported that the use of platelet enriched fibrin glue, a similar preparation to MPM, results in a statistically significant amount of bone gain. This result agreed with our study, where a new concept of fabricating growth factors-enriched bone graft matrix has been introduced, this offers the MPM the positional stability by stabilizing the bone particles, preserving its shape with subsequent “in situ” immobilization of the component of ridge preservation materials and this was the logical evolution of the fibrin glues function to incorporate platelets within the products (28).

The current study revealed the osteo-conductive potentials of both materials. In this study the newly formed bone was assessed histologically and histomorphometrically, and from the statistical analysis of the histomorphometric results, MPM showed better quality...
of bone formation regarding the homogeneity and the increased amount of the newly formed bone.

However, an interesting finding was that although the study group showed to be more significant in bone density, but the change in the mean percentage of bone was not statistically significant comparing the two groups demonstrated by p value (p=0.008). This means that the mean bone density is significantly higher in the study group but the pattern of improvement is nearly the same throughout time comparing the two groups. This could be attributed to the fact that the fibrin matrix in which the bone graft is suspended does not have any osteoconductive properties by its own, but the mineral fraction is the one that gives such property.

These findings agreed with Dr. Perissé (29) in its histological studies revealed the presence of monocytes inside the MPM biopsies, these monocytes are very important in bone formation as they allow a regulation of production of BMPs which are highly important proteins in the induction of bone production.

Also, MPM is rich with growth factors EL Moheb (21) claimed that the MPM is the only natural and autogenous product that can offer stability to bone graft particles. He conducted a study and compared MPM to PRF and favored MPM biologically and histologically, which again was proved by our results but compared with bone graft instead revealing higher regenerative features of MPM.

Our study supported Choukroun et al. (11) results who reported a 30% decrease in the healing time needed for sinus floor augmentation prior to dental implant placement when the graft material is used in combination with plasma concentrates. The authors attributed this accelerated healing to the fibrin network and platelets growth factors. This could explain the stability of the graft volume after loading in the study group compared to the control group.

Also during manipulation, the retention in the fibrous mesh of the bone fragments or the grafting material conserves its cohesion and avoids its departure away from the recipient bed.

The use of MPM in periodontology and implant therapy has a great impact in the outcome of the grafting surgery because it enhances transport of hemostatic by securing its implementation as explained before by Mazzoni and Perisse (22).

Therefore from this study we found that the use of bone filler combined with fibrin, platelets and leukocytes in the form of MPM shows a better histological evidence of hard bone formation as evidenced by its high osteoblastic activity and maturation, also proved by the histomorphometrical results regarding the higher p value in the MPM group.

**CONCLUSION**

Within the limitation of our study we concluded that:

1. Both MPM and Bone graft induced new bone formation, however, MPM possesses osseconductive as well as osseointuitive abilities.
2. MPM not only enhances the rate of new bone formation, but also increases the quality (density) of the newly-formed bone.
3. MPM can be very effective for reconstruction of bony defects.
4. MPM reduced dispersion of the bone graft particles which may act as an aid in reducing the amount of the needed bone graft.
5. Mineralized plasmatic matrix is a cost-effective source of growth factors and is easy to prepare.

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**


