Technical Note

Mineralized Plasmatic Matrix to Enhance the Bone Grafting Technique

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(Accepted for publication, March 28, 2017)

Abstract: Dental implants are the best choice to replace missing teeth. However, to place an implant sufficient bone around the implant is needed. Sometimes, the height or the thickness of the natural bone where an implant should be placed is not sufficient. For such cases bone grafting is recommended. In order to succeed bone grafting, it is necessary to achieve a good stability of the graft, enough vascularisation, and a tension free closure of the flap. The use of screwed bone block may solve the stability problem. However, it is hard to shape it, time consuming and it oblige the surgeon to open a second site to harvest the bone. Till today it is recommended to use particles in grafting for small bone defects, because the particles are not stable and it is hard to keep it in place under the chewing forces and movements. The Mineralized Plasmatic Matrix solves this problem, and opens a new age for the use of particles grafting, because by using the fibrin network, it gathers all the particles and offers a very good stability for the graft.

Key words: BPM; Platelets; Growth factors; Fibrin; Monocytes.

Introduction

Teeth’s loosening is a problem that faces a big number of the world population. Especially that the world population is going more and more (elder1), associated to a poor oral health2). Losing teeth for any reasons always follow by bone loosening. This would compromise the implant placement, or at least the implant aesthetics. The Bone grafting is the best way to rebuild a correct bone anatomy which would be able to receive a dental implant. The implant should be surrounded by at least 1 mm of bone, to be successful3). The graft is defined by placing tissue on a patient, taken from the same patient (autograft), from another patient but from the same race (human-to-human: allograft), from another race (animal to human: xenograft), or synthetic bone graft (alloplastic or biomaterials)4).

These bone grafting materials can be used as block or as particles. They all have advantages and disadvantages. Some are more difficult than others and some do not allow the promising results. Dr. Perisse, Toulouse in France, developed a new way to apply these bone grafts (all kinds of particulate bone grafts). This way is called MPM: Mineralized Plasmatic Matrix. This new promising way might facilitate the use of these bone grafts and enhances the outcomes. In our study, we are trying to investigate this enhancement - if any - in the bone grafting procedures, and the efficiency of the MPM in these surgical procedures.

We prepared with the veterinary a protocol of bone grafting cheeps, according to the Moroccan law of animal protections. Concerning the study on humans, the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation in Syria and with the Helsinki Declaration of 1975, as revised in 2000. All the patients signed also an agreement and they were aware about our study. In addition, they gave their written consents. This study can be used as a base to demonstrate the efficiency or not of the MPM compared to the classical bone grafting. Regarding definition of the MPM, the use of the particulate bone graft in implant dentistry has its limits. In fact, this kind of grafts is used in limited cases where the particulates are stable and cannot move. To secure the stability of the particles, the surgeon might use membranes. These membranes could be resorbable or not, reinforced by titanium or completely made of titanium such as the titanium mesh. The use of titanium mesh or membrane is necessary because the surgeon needs to gather the particles together and to stop the leak of the graft. Therefore, this kind of graft is used inside the socket, or during the immediate implantation or in case of small fenestration. So, the aim of the membrane is to hold the particles together.

Then MPM using the autologous Fibrin network will hold all the bone particulate together. It is prepared by drawing four tubes of 9 ml of patient’s venous blood, the tube s will be placed in the centrifugation machine, and the centrifuge is a device that allows you to settle various suspended particles in a liquid solution. These particles, due to their different sizes and in mass are deposited at different distances to the bottom of the tube and will therefore be separated at the end of the operation function to their differences4) since platelets are enucleate and the smaller blood elements5) they will therefore be in the intermediate portion (between the physiological saline and red blood cells) of the tube, and the red blood cells and will precipitate in the bottom of the tube. In fact, at the end of the centrifugation, the content of the tube can be divided into three parts:
The aim of this study was to compare the MPM grafting with the filled by bone substitute in particulate form, as a classical way. Matrix (MPM). This bone will be used to graft the created bone defects homogenous bloc (Fig. 1c). This is called the Mineralized Plasmatic coagulation reaction. And all the bone particulate will be a one and plasma mixed with sheep bone and synthetic bone substitute, starts wet Synthetic bone, and activated blood of the sheep (Fig. 1b). The collection using a syringe and added to a plastic sterile cup that contains once the centrifugation finished, the Yellow plasma (Fig. 1a) was drawn out, and centrifuged for 15 minutes at 2,500 RPM. The angulations of tubes were 30° and the distance between the rotation axes and the tubes was 4 cm. to reach a centrifuge force equal to 400g. Once the centrifugation finished, the White plasma was collected, cut in small particles containing the graft, the dense fibrin network, and the promoting healing). This procedure allows linking all the particulates together in a one product, and provides the link of the particles. This stability of the graft, open a new age of the use of particulate bone grafts.

Materials and Methods

Study number 1

Seven sheep have been chosen by the veterinary in Morocco per the Moroccan law of animal protection. 2 defects were created distally to the mandibular premolars, by using a drill. We used local anesthesia, and the sheep were calmed down by using special drugs. Operation was not performed under general anesthesia to avoid animal death risk. Once the defect was created 4 tubes of sheep’s blood of 9 ml were drawn out, and centrifuged for 15 minutes at 2,500 RPM. The angulations of tubes were 30° and the distance between the rotation axis and the tubes was 4 cm. to reach a centrifuge force equal to 400g. The plasma mixed with sheep bone and synthetic bone substitute, starts a coagulation reaction. And all the bone particulate will be a one and homogenous bloc (Fig. 1c). This is called the Mineralized Plasmatic Matrix (MPM). This bone will be used to graft the created bone defects of the sheep, in one side. On the other side, the created defects will be filled by bone substitute in particulate form, as a classical way.

The aim of this study was to compare the MPM grafting with the usual grafting used in dental surgery.

Study number 2

After collecting all the information concerning the patient, (health problems), the patients were informed about our treatment and about our protocol. They signed a consent accepting the cooperation in our study and knowing all the risks. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation in Syria and with the Helsinki Declaration of 1975, as revised in 2000.

The study contained 18 partially edentulous patients of both genders: 11 males (61.2%) and 7 females (38.9%), the age range was 39 to 43, the loss of teeth was in the posterior region of the maxilla. The 18 patients had a loss of alveolar bone height, the thickness of alveolar bone height between 3-5 mm, this makes impossible the implant placement without any bone grafting. The external sinus lift procedure was chosen (Fig. 1d). Lifting the sinus membrane and bone grafting under the membrane was performed, then the implants were placed after 14 days, at the same day of implantation a biopsy from the floor of sinus was taken, all the patients in this study accepted this protocol.

9 patients received PRF (Group 1), mixed with bone grafting and 9 received MPM (Group 2). Immediately after elevating the sinus, the blood was drawn from the patient. Either to prepare the MPM or to prepare the PRF, the MPM was prepared following the same protocol described above. While the PRF was prepared by using glass tubes, the tubes are centrifuged using the same centrifuge as for the MPM, but as the tubes are glass tube, the plasma will coagulate while it is in the tube.

The plasma will be collected already jellified and not as the plasma in the MPM in a liquid state. This gel was collected, cut in small particles and mixed with bone substitute in particulate form.

Histological Study

All biopsies were delivered in formalin; the samples were collected from the trephine and were decalcified using Nitric Acid 6% for three days. After decalcification, routine histological preparation was performed and a conventional H&E slides were studied using light microscope. Concerning cheeps 2 biopsies was done one at 14 days one at 2months. While for human a single biopsy was done during the implant placement at 14 days.

Results

Concerning study 1

At 14 days, the cut 61 is the cut with MPM (Fig. 2a) contains woven bone, osteocytes, osteoblast and fibroblast. For biopsies without MPM, we couldn’t find the particles. All the graft was disappeared; this was due to the leak of link between bone particulates. And the defect which was made by our Burr stayed the same. But we took some of the tissue to analyze it and we found osteoblasts, osteocytes, and fibroblasts. Two months later another biopsy was taken. The macroscopic aspect in both cases was looking like bone shape. But on MPM side we obtained a big thickness of bone while on the other side we could still see a defect. But a healed defect, because we lost from the beginning all the graft. While the MPM side. We could see big parts of bone, osteoblasts lining the bone that singes the new bone formation, osteocytes and fibroblasts (Fig. 2b).

Concerning study 2

Group 1:

It was filled with newly formed woven bone, osteoblast activity was clear on the rimming of the newly formed osseous islands, some osteoclast cells were located centrally (Fig. 3a).

Group 2:

It was filled with granular tissue (Fig. 3b), no evidence of bone...
We seek to isolate platelets or to concentrate the platelets of the patient because platelets are a natural source of growth factors\textsuperscript{9}. The way to isolate the platelets is to centrifuge the blood. The centrifuge is a device that allows you to settle various suspended particles in a liquid solution. Since the platelets are the smallest body and have the smallest mass of the different element of the blood, after the centrifugation, the platelets theoretically can be isolated at the upper part of the tube. The speed, the time and the tubes positions can make a big variation in the number and in the position of the platelets inside the tube. The more the speed increases or the more the speed increases the less platelets are isolated. After the centrifugation, the tube will be divided into 2 parts. The red part in the bottom of the tube that contains the Red Blood Cells, and the Yellow the upper part that contains theatrically all the platelets.

Unfortunately, this is not always true, there are a lot of variations and some platelets will migrate inside the red part of the tube. We took blood of 5 normal patients; analysed the number of platelets, it was shown that the number varies between 250 000 and 350 000 mm\textsuperscript{3}. We took 9 ml of these patients’ blood, and we centrifuged it on 2500 rpm during 12 min. At the end of the centrifugation the tube was separated into 2 parts. The yellow (part) at the top and the red (part) in the bottom were detected. The yellow (substance) was taken out to analyse the number of platelets, the results were 7000 platelets only. And the more we reduced the speed and the time the more we obtained platelets. The maximum that we obtained was at 800 rpm during 3 min it was 70\% of initial number. To prepare the PRF the used tubes should contain an anticoagulant, the yellow part is collected and centrifuged once again to concentrate the platelets. Then the platelets are collected once again and injected in the site with thrombin and calcium chloride. In Europe, this procedure is forbidden therefore we made our study on the PRF.

To prepare the PRF the used tubes are plastic tubes that contain clot activators. This will activate the intrinsic way of coagulation and the fibrinogen will be transformed into Fibrin network. At the end of the centrifugation, the yellow part will not be a liquid but it will be as a gel. This is the Fibrin of the PRF. This Fibrin should contain all the platelet factors and cytokines such as TGF, PDGFs and IGFs which plays an important role in the healing process. The platelet’s release of cytokines can stimulate the colonization and proliferation of other cells which are important for the reparation or regeneration process\textsuperscript{8,10}. We seek to isolate platelets or to concentrate the platelets of the patient because platelets are a natural source of growth factors\textsuperscript{9}. The way to isolate the platelets is to centrifuge the blood. The centrifuge is a device that allows you to settle various suspended particles in a liquid solution. Since the platelets are the smallest body and have the smallest mass of the different element of the blood, after the centrifugation, the platelets theoretically can be isolated at the upper part of the tube. The speed, the time and the tubes positions can make a big variation in the number and in the position of the platelets inside the tube. The more the speed increases or the more the speed increases the less platelets are isolated. After the centrifugation, the tube will be divided into 2 parts. The red part in the bottom of the tube that contains the Red Blood Cells, and the Yellow the upper part that contains theatrically all the platelets. Unfortunately, this is not always true, there are a lot of variations and some platelets will migrate inside the red part of the tube. We took blood of 5 normal patients; analysed the number of platelets, it was shown that the number varies between 250 000 and 350 000 mm\textsuperscript{3}. We took 9 ml of these patients’ blood, and we centrifuged it on 2500 rpm during 12 min. At the end of the centrifugation the tube was separated into 2 parts. The yellow (part) at the top and the red (part) in the bottom were detected. The yellow (substance) was taken out to analyse the number of platelets, the results were 7000 platelets only. And the more we reduced the speed and the time the more we obtained platelets. The maximum that we obtained was at 800 rpm during 3 min it was 70\% of initial number. To prepare the PRF the used tubes should contain an anticoagulant, the yellow part is collected and centrifuged once again to concentrate the platelets. Then the platelets are collected once again and injected in the site with thrombin and calcium chloride. In Europe, this procedure is forbidden therefore we made our study on the PRF.

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**Discussion**

This work initially was initiated using growth factors, in dental implant treatment. The use of growth factors was introduced for the first time in 1974 by Ross et al.\textsuperscript{11} who was the first to describe a growth factor from platelets. Based on Tayaponsak protocol, Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) has been developed\textsuperscript{8}. These techniques are based on the idea of concentration of platelets for use as a drug in some serious diseases\textsuperscript{9}. These both products the PRP and the PRF were used in dental implant therapy either to accelerate the healing or to help regenerating bone where needed. The use of growth factors was introduced for the first time in 1974 by Ross et al.\textsuperscript{11} who was the first to describe a growth factor from platelets. Based on Tayaponsak protocol, Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) has been developed\textsuperscript{8}. These techniques are based on the idea of concentration of platelets for use as a drug in some serious diseases\textsuperscript{9}. These both products the PRP and the PRF were used in dental implant therapy either to accelerate the healing or to help regenerating bone where needed. The PRP or PRF preparation needs to centrifuge the patient blood, to separate the plasma (PRP) and the platelets. Then the platelets are collected once again and injected in the site with thrombin and calcium chloride. In Europe, this procedure is forbidden therefore we made our study on the PRF.

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bone particles inside. So, if the PRF should be used in the bone grafting, it should be cut in small particles and then mixed with the bone or the bone substitute. This technique does not offer a homogenous structure\(^8\). The MPM is prepared by a single spin using empty tubes without neither anticoagulant nor clot activator. At the end of the centrifugation, the superior part of the tube will contain the fibrinogen, platelets, and monocytes\(^11\). This part stayed in liquid, which permit the mixture with the bone. This liquid will be collected and added to the bone graft, or the bone substitute before it coagulates. Once this plasma is in contact with the calcium of the bone graft, the activation will start and the transformation of the Fibrinogen into fibrin network will begin. This characteristic is very important because it allowed us to obtain a one homogenous component which contains the bone graft inside, the fibrin network, the growth factors, and cells. This is all the importance of the MPM. In our studies, on animals as shown by the results, from the MPM side, the graft stayed there. And during the biopsies we could collect the graft after 14 days. The histological analysis showed high cell activity, while from the bone substitute. We couldn’t find anything left, because of the mobility of the animal, who is going to chew all the day. The stability of the graft is one of the most important conditions for the success of the bone grafting. This stability is offered by the MPM compared to the bone substitute alone. In our study on humans, the side that contained MPM showed woven bone on 14 days while the side of the PRF which was mixed with bone contains only granulation tissue. This can also be explained by the stability of the graft. In fact, to have a bone remodelling, or regenerating, a space should be motioned and the scaffolding should be secured. The PRF alone is not able to preserve the space necessary for the bone formation because since it is a gel it will not be able to resist to the chewing forces. It is true that it gives the necessary extracellular matrix needed by the cells, but since the space is not preserved, the bone cannot be build. Therefore, the need to use the bone graft or the bone substitutes to secure the scaffolding. But since the bone particles and the PRF are not linked together (Fig. 4a)\(^{10}\), there is no stability of the graft. Since the life time of the platelets is only 8 days and since the bone remodelling is more than 9 days. So there is no interest to cut the PRF and to mix it with the bone graft. It will not give the stability to the bone and it will not give enough growth factors to help in the bone regeneration. While Since the MPM shows a sticky and homogenous component. So, once the bone particles or the bone graft is placed on the site, it will stick to the site. Therefore, the fibrin act as fibrin glue and this way the whole mass of the MPM will not move. This will keep the spaces which are for the bone formation. In addition to the bone particles are linked together by a strong fibrin network. So, the scaffolding is secured (Fig. 4b, c)\(^11\). Since the stability and the scaffolding are secured by the MPM, this will play a very important role in the bone regeneration. In addition to that, the MPM contains some cells such as platelets which add the growth factors or the cytokines, and the monocytes that play an important role in the regulation of the natural BMP3,4,11.

To succeed the bone grafting, several conditions should be fulfilled, the space maintaining, the scaffolding, the stability of the graft and the good closure. The concentration of platelets will play a small role in the bone regeneration. Since the life time of platelets is only from 4 to 8 days. So, few days after the concentrated platelet is placed, their concentration will decrease dramatically to reach the normal concentration needed by the human body. So, what should be taken in consideration in these products is neither the biological part, nor is the presence of cells, but the important thing in these products the biomechanical part. The MPM is the only natural and autogenous product that can offer the stability to the bone particles. This stability was missed in the particles on prepared as MPM. The use of the PRF alone or mixed with bone graft or bone substitute, will not give the needed stability or the needed resistance to the chewing forces, so will not help in the bone regeneration. Based on the structure of the MPM, this product should be considered every time a bone grafting procedure is approached.

Conflict of Interest

The authors have declared that no conflict of interest.

References