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Combination of Nanocrystalline Hydroxyapatite and Injectable Platelet-Rich Fibrin on Bone Graft Materials for Alveolar Bone Preservation

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Abstract

Alveolar bone resorption is one of post-extraction complications with a reduction in the dimensions and quality of the alveolar bone, which will make it challenging to install dental implants in the future. The resorption can be prevented by preserving the alveolar bone using bone grafts. Nanocrystalline hydroxyapatite (HA) is a widely developed material as a bone graft. However, there are still some limitations because it only has osteoconductive properties. The addition of injectable platelet-rich fibrin to HA can increase this material's osteoinductive, antibacterial, and anti-inflammatory properties, making it suitable for use as bone graft material for the preservation of alveolar bone.

Introduction

Alveolar bone resorption is one of the complications post-extraction where there is a reduction in the dimensions and quality of the alveolar bone. This alveolar bone resorption will significantly complicate the rehabilitation phase of tooth loss and post-extraction especially during the installation of dentures such as dental implants. Dentures require placement in an excellent three-dimensional location in the oral cavity. Alveolar bone resorption is known to occur over time, but resorption in the first few months of tooth extraction is known to be very significant [1]. The systematic review conducted by Tan *et al.* (2011) showed that horizontal bone resorption reached 29–36% and vertical bone resorption reached 11–22% in the first 6 months after tooth extraction [2]. The review suggests that the alveolar bone resorption prevented and treated correctly post-tooth extraction would adversely impact [3].

Tissue engineering techniques to accelerate healing and prevent excessive resorption of alveolar bone are also known as alveolar bone preservation. Preservation alveolar bone generally uses a bone graft material (bone graft) that benefits new bone formation, thus preventing excessive resorption of alveolar bone. Bone graft materials used in the preservation of alveolar bone are still a topic that has been extensively researched to date to obtain effective materials [1], [4], [5], [6].

Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] is a bone graft material that has good biocompatibility and can form strong chemical bonds with bone tissue. This material is also known to have biointegration and osteoconductive properties [7], [8], [9]. The nanocrystalline form of HA (10–100 nm) closely resembles natural HA in bone. It has close contact with the surrounding tissue, resorption properties, and a high number of molecules on the surface [10].

The ideal bone graft material should have osteoconductive, osteogenic, and osteoinductive properties and mechanical stability and be free from disease. Osteoconductive is a physical property of a material that provides a framework for the growth of blood vessels and osteoprogenitor cells in bone formation. Osteoinductiveness is the ability to stimulate stem cells to differentiate in mature cells through stimulation by local growth factors. Osteogenicity is the

ability to differentiate into osteoblasts in the presence of bone growth factors in bone grafts [11], [12], [13].

Nanocrystalline HA is osteoconductive but has inferior osteoinductive properties; therefore, one of the efforts to increase the ability of this material is to combine it with platelet-rich fibrin (PRF). PRF is a second-generation platelet concentrate introduced by a French researcher named Choukroun in 2001. This biomaterial is extracted from the blood autogenous and has been widely used to promote the formation of soft and hard tissues. PRF is rich in cytokines and growth factors that can increase cell proliferation and differentiation, increase angiogenesis, act as a matrix for tissue growth, regulate inflammatory, and anti-infective reactions. Growth factors from PRF such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin growth factor (IGF) are known to play a significant role in healing and bone regeneration to prevent excessive alveolar resorption after tooth extraction [14], [15], [16].

The form of PRF that has excellent potential for tissue healing is injectable-platelet-rich fibrin (i-PRF). i-PRF is a PRF formed through the concept of low-speed centrifugation, where the PRF formed is in the form of a liquid that can be injected. i-PRF contains more platelet cells, growth factors, and leukocytes than conventional PRF. i-PRF is also known to have antibacterial and anti-inflammatory effects [17], [18], [19], [20], [21], [22], [23].

The development of new biomaterials as bone grafts is a topic that is still being studied at this time to find biomaterials that are effective, inexpensive, and easy to obtain. A good bone graft must provide not only osteoconductive properties but also biocompatible and good mechanical properties. It should be able to be used as a delivery system that carries growth factors, anti-inflammatory, antibacterial, and antiosteoporosis [24].

Alveolar Bone Preservation

One form of tissue engineering technique to prevent excessive resorption of alveolar bone is the alveolar bone preservation procedure. Preservation of alveolar bone after a tooth extraction is a procedure performed immediately or sometime after tooth extraction which aims to minimize alveolar bone resorption and maximize alveolar bone growth [3], [25], [26].

In some cases, it is impossible to preserve the alveolar bone immediately after extraction due to acute infection; therefore, preservation is carried out several weeks after extraction. Alveolar bone preservation can be done with several techniques such as minimizing trauma during extraction, soft- and hard-tissue grafts, the use of membrane barriers, a combination of these techniques, and other methods [3], [25], [26].

Preservation of alveolar bone using a bone graft material (bone graft) is known to have benefits in new bone formation, thus preventing excessive resorption of alveolar bone [1], [4]. The meta-analysis research conducted by Bassir *et al.* (2018) concluded that the preservation of alveolar bone using bone graft material effectively minimizes the reduction in the vertical and horizontal dimensions of post-extraction alveolar bone [4]. Alveolar bone preservation using bioactive ingredients is also expected to accelerate the time required for post-extraction bone healing for prosthodontic purposes [27].

Bone Graft

Bone graft is a bone replacement material used in surgical procedures to replace the lost bone with materials from the patient's own body, other people's bodies, natural substitutes from different species, and synthetic materials to help reconstruct, stabilize the structure, and bond to the bone, and stimulate the process of osteogenesis, and healing of bone defects.

The function of bone graft in biomedical applications is to stimulate fracture healing, stabilize dental implants, stimulate healing of two diseased joints, regenerate bone lost due to trauma, infection, or other diseases, improve bone healing response, and regenerate bone tissue around the implanted device, for example, replacement. Artificial joints and bone grafts are also used as plastic arthrosis acetabulum (congenital dislocation of the hip or Perthes disease) [28].

The physiological properties that bone graft must possess are osteogenesis, osteoinduction, and osteoconduction. Osteogenesis is the ability of the bone graft to produce new bone and this process depends on the living bone cells in the graft. The osteogenic material graft contains cells that can form bone (osteoprogenitor cells) or have the potential to differentiate into bone-forming cells (induced osteogenic precursor cells). These cells play a role in the early stages of the healing process to fuse the graft with the bone host. Osteogenesis is a trait found in autogenous and bone marrow cells [13], [29].

Osteoinduction agents are generally proteins that induce stem cells that do not differentiate into osteogenic cells or generate stem cells to increase [13]. The physiological feature of bone graft next is that osteoconduction occurs when the bone graft material serves as a scaffold for new growth that is perpetuated by the original bone. Osteoblasts from the grafted defect border utilize material bone graft as a framework to emerge and produce new bone. At least the bone graft material must be osteoconductive [13], [28].

The bone graft can be classified into four types based on the source, namely:

a. Autograft or autogenous bone graft is bone tissue derived from the individual itself. It is the gold standard with osteogenic, osteoinductive, and osteoconductive properties. These grafts are usually obtained from the maxilla, mandible, skull, tibial plateau, iliac crest, and ribs. Bone graft does not elicit a rejection response from the patient's immune system because it comes from the patient's own body [6], [13].

b. Allograft tissue osseous non-vital taken from one individual and transferred to another individual of the same species. Allografts are usually taken or obtained from cadaver who has donated bone so that it can be used for people who need it. Usually, the graft is obtained from a bone bank. Oral surgeons usually use this material as a second alternative to grafts autogenous [6].

c. Xenograft or heterograft, derived from species other than humans, such as cattle. Xenografts are usually distributed only as a calcified matrix [6].

d. Alloplastic is a synthetic material developed to replace human bone. Some alloplastics are made of calcium phosphate (CaP), ceramic HA other, biphasic CaP, tricalcium CaP, calcium sulfate, and calcium biocompatible composite polymers [6], [13], [28].

The healing process of the bone graft union with the bone has become host similar to the bone healing. The success of a bone graft depends on the recipient site, local growth factors of the host, viability of the bone graft, the volume of the grafted bone, and the structural function of the bone graft. The number of osteoprogenitor cells and the quality of the connective tissue vasculature host determine the ability to respond to BMPs and other growth factors of bone grafts. Factors that can affect the fusion of bone grafts are the type of trauma, infection, blood vessel supply, and the stability of the bone injury. The instability of bone grafts, host bone, and soft tissue will inhibit revascularization [30].

The bone graft will produce a response to the accumulation of inflammatory cells, followed by a chemotaxis process of host mesenchymal cells in the grafted area. The following process is differentiating primitive cells from the host into chondroblasts and osteoblasts. This process is influenced by various osteoconductive factors [11], [31].

The following process will be revascularizing the bone graft and necrotic bone resorption. Eventually, bone is formed from osteoblasts in the grafted bone, followed by a remodeling process (Figure 1). The bone graft of dental implant is a material that encourages the new bone formation through osteogenic, osteoinductive, or osteoconductive processes. Ideally, the natural bone graft will undergo resorption and be replaced by one from the host. The time required for bone graft resorption varies greatly depending on the type of bone graft used [11], [26], [29], [31], [32].

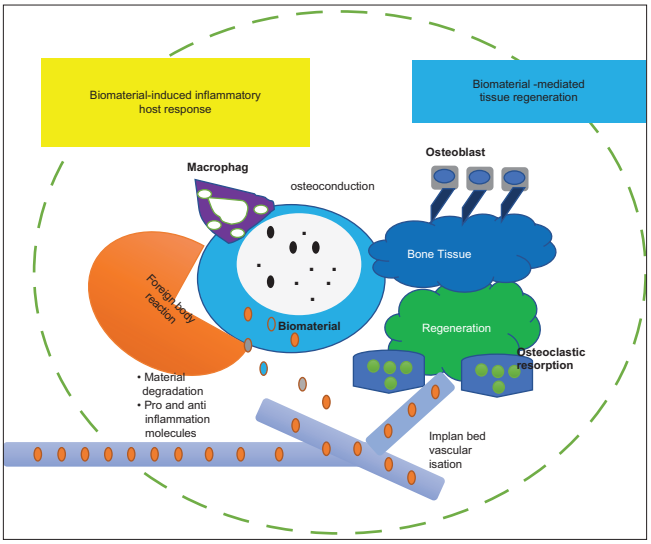

Figure 1: Theoretical pathway between bone graft biomaterials with inflammation respons of the host and its relation with the tissue regeration [31]

HA Nanocrystalline

HA is a calcium compound in the form of crystals and has the chemical formula [Ca10(PO_4)6($OH)_2$]. The HA composition by weight ratio in bone is 60%, in dentin 70%, and enamel 96%. Synthetic HA has a chemical composition similar to inorganic bone tissue. HA crystals are found in bone, dental hard tissue, enamel, and dentin in humans and animals [7].

HA is one of the CaP groups that is used in biomedical applications. The stability of the CaP composition is regulated by the ratio of calcium to phosphate, the amount of water content, the processing temperature, and the pH of the surrounding environment. Several phases of CaP used in biomedical applications include amorphous calcium phosphate (ACP), octacalcium phosphate (OCP), three calcium phosphate (or TCP), and HA. The solubility level of several CaP phases under physiological conditions is ACP > TCP > TCP > HA. HA is the most stable phase among the three other CaP phases under physiological conditions [24].

HA has a hexagonal P6space $group_3$/m, with basal plane edge a = 0.9432 nm and height c = 0.6881 nm. This space is filled with the formula [$Ca_{10}(PO_6)_3(OH_2)$]. The hydroxyl ions are projected at an angle rhombic, which is half the height of the space unit. The six calcium ions associated with this hydroxyl form an equilateral triangle centered on a perpendicular hydroxyl column. In general, the calcium triangles

are sequentially rotated at 60°C. The four calciums run along with two parallel columns that separate into the hydroxide column by half the height of the calcium triangle. These calcium ions are associated with oxygen from the orthophosphate tetrahedra. The hydroxyl atoms of oxygen are 0.03 nm apart from the plane of the calcium triangle. Hydroxyl leads to oxygen-hydrogen bonds along the column axis but not across the plane of the calcium triangle [33].

HA has good biocompatibility and the ability to form strong chemical bonds with bone tissue. HA can remineralize bone tissue that is lost or damaged without causing a rejection reaction by the body. In solid and solid form, HA in tissues is very stable and insoluble in acidic environments [7], [24].

The mechanism of HA can accelerate bone healing, namely, when implanted into bone defects, HA will release CaP so that it increases body fluid saturation and precipitates biological apatite in the area. This apatite may contain endogenous proteins and act as a matrix for osteogenic cell attachment and growth [34].

The nanocrystalline form of HA (10–100 nm) is known to have better bioactivity than coarse crystals. The attachment of osteoblasts and osteoclasts is known to occur more in nanocrystalline HA than conventional HA [35]. Pascawinata *et al*. (2013) study showed more and faster bone healing in defects implanted using nanocrystalline HA than conventional HA and defects left blank [36]. The advantages of nanocrystalline HA as bone graft material are its closer contact with the surrounding tissue, its faster absorption, and the high number of molecules on its surface. Synthetic nanocrystalline HA shows good biological properties such as biocompatibility, bioactive, non-toxic, does not cause inflammatory and immune reactions, and bioresorbable [10], [37], [38].

Pezzatini *et al*. (2005) concluded that nanocrystalline HA could improve endothelial cell function during angiogenesis and is very well used as a pro-angiogenic agent. The process of angiogenesis is a prerequisite for osteogenesis [39]. The study by Kasaj *et al*. (2008) concluded that nanocrystalline HA in the form of a paste can stimulate the proliferation of human periodontal ligament cells [10].

The development of science and technology has led to more research on HA. However, it is difficult to obtain pure properties because CaP has many derivatives, and the synthesis of CaP is highly dependent on the reaction conditions and the ratio of moles of Ca/P. Various techniques can be used for the manufacture of HA powder, according to the several kinds of the literature. Numerous methods can be used to synthesize nanocrystalline HA, including the process of sol-gel, precipitation, batch hydrothermal, microwave irradiation, mechanochemical, chemical vapor deposition, biomimetic, and others [8], [40], [41], [42].

There are two main ways to make HA powder: The wet method and the solid reaction method. The production of HA using the wet process can be further divided into three groups, namely, precipitation techniques, hydrothermal techniques, and hydrolysis of other types of CaP derivatives. The degree of crystallinity depends on the technique, material with various morphologies, and stoichiometry. Solid-state reaction techniques usually give products that are stoichiometric and well crystalline, but require relatively high temperatures and long heating times. In addition, the sintering ability of the obtained material is usually low [8], [41].

Hydrothermal techniques usually provide HA materials with good crystallinity and a Ca/P ratio close to stoichiometric values. The crystal size is in the nanometer to millimeter range. Hydrolysis technique can make HA by hydrolysis of TCP, monetite, brushite, or OCP at low temperature to produce HA crystals with micron size but the product is not very stoichiometric. Each HA manufacturing technique has advantages and disadvantages [8], [41].

PRF

PRF was first developed in France by Choukroun and Ghanaati. which is used to promote healing of soft and hard tissues. PRF is superior to platelet rich plasma, where preparation is easier and does not require chemical manipulation of blood [17], [43], [44]. PRF is a fibrin matrix in which cytokines, growth factors, and cells are trapped. This material can be used for the reconstruction of various defects. Making PRF only requires centrifuged blood without the addition of chemicals such as anticoagulants, bovine thrombin, and calcium chloride. Making PRF is not only easy to manufacture and also relatively inexpensive [43].

The advantages of PRF are its ease of preparation and ease of application, low cost, and lack of biochemical modifications so that no bovine thrombin or anticoagulants are required. This advantage greatly reduces the biochemical treatment of blood as well as the risks associated with the use of bovine-derived thrombin [45], [46]. The weakness of PRF is in its preparation and storage. PRF will provide good clinical benefit, depending on the time interval between the speed of handling blood collection and centrifugation as PRF prepared without anticoagulant. Another disadvantage of PRF is its storage after preparation, where the PRF membrane must be used immediately after preparation because if it is not used immediately, it will shrink and will cause dehydration which will change the structural integrity of the PRF. This dehydration will also decrease the content of growth factors in the PRF. If placed in the refrigerator, PRF can cause the risk of bacterial contamination of the membrane [47].

Clinical studies reveal that this biomaterial can be a favorable matrix for developing a coherent cure.

The concentrate in the form of a platelet gel can be used in conjunction with bone grafts which has several advantages, such as accelerated wound healing, bone growth and maturation, hemostasis, provides better case handling for the graft material, and can also be used as a membrane. Many clinical trials suggest a combination of bone graft and PRF to increase bone density [45].

Several clinical studies have shown varying results when PRF is applied as a material used for the preservation of tooth sockets after tooth extraction. Research Ezirganli *et al*. (2014) showed that the use of PRF alone as bone-guided regeneration without the use of bone graft did not show significant improvement because it only made a small increase in bone; therefore, it was recommended to use it together with material guided bone regeneration, while the study of Srinivas *et al*. (2018) showed that PRF can improve wound and bone healing after tooth extraction [48], [49]. The variation in the results of these studies may be based on protocols that differ in conditions and in each study, and until now, clinical and laboratory studies are still being carried out to achieve maximum results in the treatment.

Research on the topic of PRF continues to this day. Choukroun and Ghanaati introduced a “low-speed concept” for centrifugation of blood in the process of making PRF where at a low-speed centrifugation showed a higher number of leukocytes, platelets, and growth factors than standard PRF [17], [50]. Joseph Choukroun then classified the manufacture of PRF with the low-speed concept, namely:

1. A-PRF (advance PRF) = 1300 rpm/14 min
2. A-PRF (advance PRF+) = 1300 rpm/8 min
3. i-PRF = 700 rpm/3 min
4. i-PRF Male = 700 rpm/4 min
5. i-PRF+ = 700 rpm/5 min
6. A-PRF liquid = 700 rpm/3 min production

The available PRF varies based on three variables, namely:

1. Relative centrifugation force
2. Centrifuge speed
3. Centrifuge time [51].

PRF acts by degranulating granules in platelets containing growth factors. These growth factors present in platelets play an essential role in cell regeneration. Growth factors released from activated platelets are shown in Figure 2.

PRF concentrate formed consists of platelets, cytokines, and a fibrin matrix. Platelets, leukocytes, and cytokines play an important biological role. Degranulation of platelets releases cytokines, which can trigger cell migration and proliferation of the fibrin matrix, beginning the first phase of the healing process. The fibrin matrix will support these elements and can be said to be responsible for the therapeutic process of PRF [47].

Fibrin is an activated form of fibrinogen present in plasma or granules of platelets and plays an important role in platelet aggregation and hemostasis. Natural polymerization of fibrin is formed during the preparation of PRF (centrifugation). The fibrin matrix formed in PRF is flexible, elastic, and very strong. This flexible fibrin matrix has the ability to support cytokines and cell migration. This results in an increase in the lifespan of the released cytokines and their use in matrix remodeling. Cytokines are needed to trigger healing [16], [52], [53].

i-PRF

Several platelet concentrates continue to develop as studies develop with various manufacturing protocols. Choukroun and Ghanaati introduced the concept of low-speed centrifugation to produce i-PRF where pembuatanya not require substances additive such as anticoagulants and can be found in the blood centrifugation at low speed of 700 rpm. The PRF formed is in the form of a liquid that can be injected. i-PRF contains more platelet cells, growth factors, and leukocytes than conventional PRF. i-PRF will coagulate a few minutes after being injected, in addition to improving healing, i-PRF is also known to contain more antibacterial effects than other platelet concentrates and also has potential as an anti-inflammatory [17], [18], [19], [21], [22], [23]. i-PRF has bactericidal and antibiofilm properties so that it can act as an antimicrobial peptide and a potential bioactive agent to prevent infection in the post-operative area [20].

Several studies have shown the success of using i-PRF in clinical applications. Research Aydinyurt *et al*. (2020) showed that the application of i-PRF was effective in reducing bone loss, modulating the inflammatory process, and influencing cytokines in periodontitis rats [54]. The study of İzol and Üner (2019) showed a positive effect of i-PRF surgery free gingival graft as a root coating of teeth in cases of gingival recession [55].

The Combination of Nanocrystalline HA with PRF

Bone graft material nanocrystalline HA is osteoconductive but not osteoinductive; therefore, one effort to increase the ability of this material is to combine it with PRF. PRF as blood cloth which is a biological product of the body can fill the empty space in bone graft implantation. The combination of bone graft and PRF has benefits such as facilitating the placement of bone grafts in place, providing stability to the bone graft material, playing a role in hemostasis, accelerating wound healing, and increasing bone growth and maturation [56], [57].

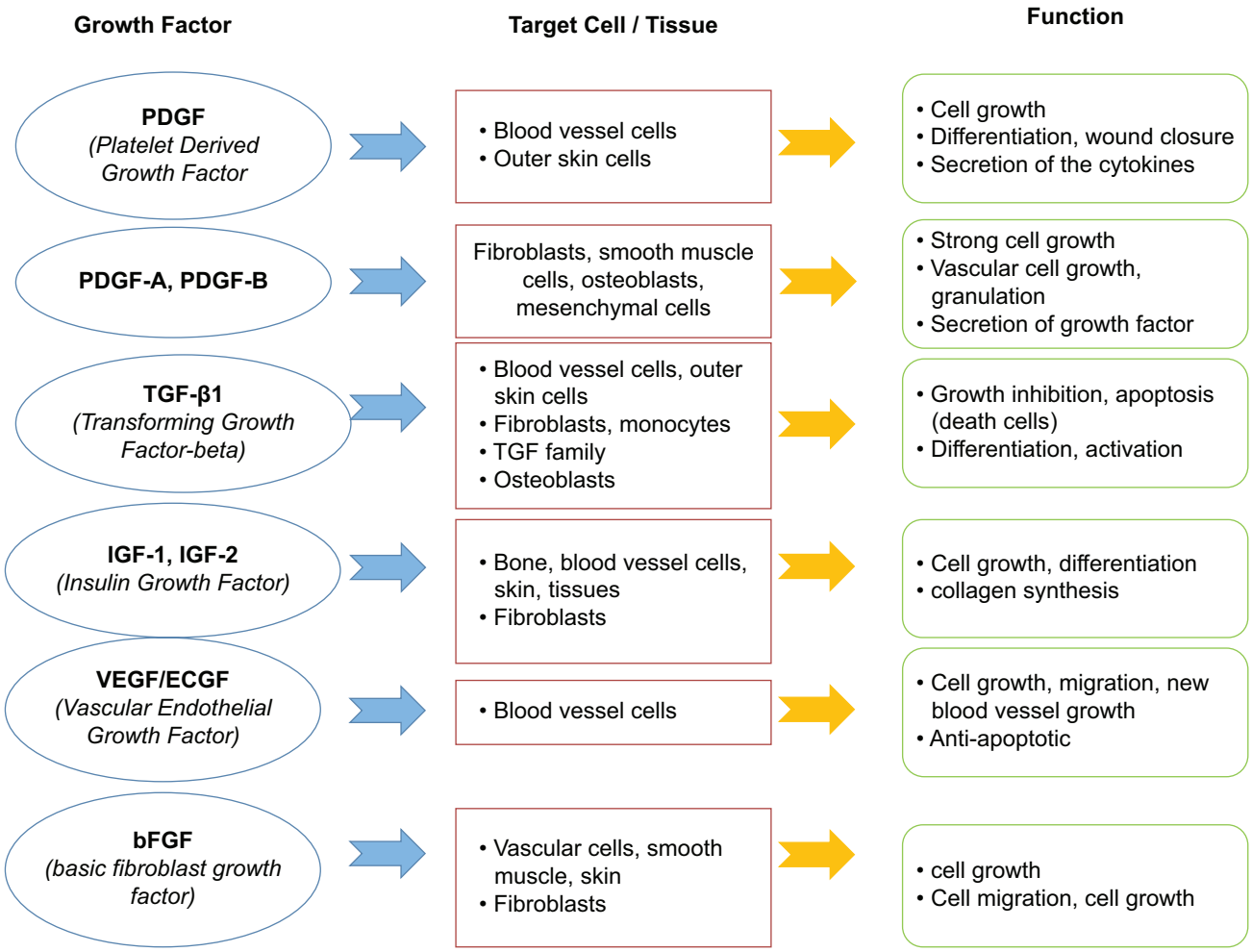

Figure 2: Growth factors on PRF [52]

Several studies have shown good results with the use of a combination of HA and PRF. Research by Deenadayalan *et al*. (2015) showed that the combination of HA with PRF can increase the acceleration of bone regeneration as evidenced by radiographic observations 1 year after implantation of the combination of HA and PRF in three cases of periapical lesions in the alveolar bone [56]. Attia's research (2015) showed the success of the combination of nanocrystalline HA in the treatment of Class II lesion defects with increased bone height in periodontal tissues [58]. Research Panda *et al.* (2013) and Anitha *et al*. (2017) demonstrated efficacy in the treatment of aggressive periodontitis [59], [60]. Research by Pradeep *et al*. (2016) showed the success of bone formation in the bone cavity due to cysts [61]. The combination of nanocrystalline HA granules with PRF can fill the cyst cavity as evidenced by radiological observations 6-month–1-year postoperatively.

Nanocrystalline HA and PRF have the ability to enhance bone healing. Nanocrystalline HA has osteoconductive ability while PRF contains many growth factors such as PDGF, TGF, and IGF which play a major role in bone healing. Research by Graham *et al*. (2009) showed that *in vivo* application of PDGF enhances bone regeneration in calvarial defects where a bioresorbable membrane is used as a carrier [14]. Research Lind *et al*. (1993) showed that TGF increases callus formation around the osteotomy area performed in rabbits, when TGF and PDGF are combined and can also increase cell growth in callus tissue so that the combination of several growth factors has potential to improve bone healing [62]. IGF-I is a growth factor secreted by osteoblasts and growth factor; this can stimulate bone formation through proliferation and differentiation when working with PDGF and TGF, although such systematic use of IGF alone is not ideal because IGF also promotes osteoclast formation and bone resorption [63]. Research Chenchev *et al*. (2017) demonstrated success in alveolar bone augmentation using a combination of bone graft material and i-PRF [64].

Socket Healing after Tooth Extraction and Bone Graft Implantation

The bone healing process in biomaterial implantation after tooth extraction follows the post-tooth

extraction healing procedure and cellular interactions begin immediately after the biomaterial is implanted. Healing in post-extraction sockets consists of four phases, namely, the hemostasis and coagulation phase, the inflammatory phase, the proliferative phase, and the modeling and remodeling phase. Hemostasis and coagulation phases occur immediately after tooth extraction. The tooth socket will fill with blood due to microvascular damage and the bleeding process. Particles bone graft impregnated into the tooth socket will be trapped in the fibrin mesh. Blood clots and platelets, besides having a hemostatic function, also play a very important role in tissue healing due to the presence of many cytokines, chemokines, interleukin families, and many growth factors [65], [66].

The inflammatory phase begins from the time the wound occurs until about the 5th day after extraction. Leukocytes play a role in debridement of bacteria and necrotic tissue. The presence of neutrophils increases the activity of osteoclasts which will erode the surface of the bone wall of the post-extraction tooth socket. Neutrophils in the wound area in a relatively short time (24–48 h) will then be in the leg by monocytes. Monocytes turn into macrophages in the next 48–96 h. Macrophages are responsible for the continuation of phagocytosis and effectively release growth factors (TGF-β, FGF, and EGF) which then activate the presence of fibroblasts and osteoblasts. Migration of inflammatory cells and osteoclasts on the surface bone graft will occur during this phase and lead to minimal and slow removal of the bone graft [66].

The proliferative phase is characterized by filling the tooth socket with granulation tissue (macrophages, fibroblast matrix, and new blood vessels), mature collagen, and osteoblasts. New woven bone formation can be detected 14 days after tooth extraction, where the most of the tooth sockets contain a temporary matrix. Mineralized bone formation also begins to appear on day 14. About 1 month after extraction, the most of the tooth sockets will be filled with lots of new woven bone containing lots of primary osteons. This woven bone will appear to be in contact with the old bone on the socket wall. The presence of bone graft in this phase can increase the growth of new bone [66], [67].

The final phase of the post-extraction healing socket includes modeling and remodeling. Modeling is changes in bone structure and tissue that occur with architectural and shape modifications while remodeling occurs without architectural and shape modifications. The remodeling phase is characterized by intramembranous ossification of the newly formed tissue, thereby filling the tooth socket with new bone. The modeling and remodeling processes are the result of interactions between osteoblasts and osteoclasts which are highly modulated by the presence of several factors such as macrophage colony-stimulating factor, receptor activator of nuclear factor kappa B, receptor activator of nuclear factor kappa B ligand, and osteoprotegerin [66].

Conclusion

The combination of nanocrystalline HA with i-PRF has potential as a bone graft material for preserving alveolar bone so it is expected to overcome the problem of alveolar bone resorption after tooth extraction. The addition of i-PRF to nanocrystalline HA can enhance this substance's osteoinductive, antibacterial, and anti-inflammatory properties.

Author Contributions

A.P conceived and designed the work; A.P. and AB organized the paper. All authors discussed the review and contributed to the final manuscript. All authors have read and agreed to the published version of the manuscript.

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