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POTENTIAL APPLICATIONS OF HYDROXYAPATITE-MINERALIZED-COLLAGEN COMPOSITES AS BONE STRUCTURE REGENERATION: A REVIEW

K. Saptaji[1,*], *D.C. Hardiyasanti*[1,2], *M.F. Ali*[1,2], *R. Frandito*[1,2], *T.K. Dewi*[1,2]

[1] *Department of Mechanical Engineering, Faculty of Engineering and Technology, Sampoerna University, Jakarta, Indonesia*
[2] *Department of Aerospace and Mechanical Engineering, The University of Arizona, Tucson, AZ, 85721, USA*
**Email: kushendarsyah@sampoernauniversity.ac.id*

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ABSTRACT

Composite materials are known for their flexibility due to the combinations of two or three different materials and manipulation of their compositions. The advantage offered by composite materials makes them suitable for biomedical applications, especially for implants. There are three types of composites biocompatible materials, namely Metal Matrix Composite (MMC), Ceramic Matrix Composite (CMC), and Polymer Matrix Composite (PMC). The biocompatible composite materials can be produced in various manufacturing processes. The manufacturing processes of MMCs are stir casting and powder metallurgy; the typical manufacturing process for CMCs is powder metallurgy; 3-D printing by synthesizing and cross-linking the networks is used for fabricating PMCs. One of the promising biocompatible composites is Hydroxyapatite Mineralized Collagen (HMC). The HMC is used to create a bone scaffold in bone regeneration. The suggested manufacturing process for HMC is a hybrid process that collaborates Additive Manufacturing and CNC Machining. This paper reviews the HMC, especially its properties, fabrication method, and existing experimentation. In addition, the three types of biocompatible composites are also discussed in the applications and their manufacturing processes.

Keywords Hydroxyapatite-Mineralized-Collagen; Tissue Engineering; Biomedical; Biocompatible Composites; Bone Scaffolds; Powder Metallurgy
Paper type Review Paper

INTRODUCTION

Biocompatible materials or biomaterials are natural or synthetic materials purposed to substitute any organ or tissue in living creatures [1]. Biomaterials have been widely used in medical industry as implants. In addition, biomaterials can also be used for augmenting and treating a particular part of the body for a period of time. The human body can recognize foreign objects hence the reaction of the body differs depending on whether the foreign objects are harmless or not. The biocompatible materials that will be implemented in the body need to be accepted and adopted. The implants cannot contain any harmful substance for the body for a short and long period. The materials also need to be adequate depending on the body part where the material will be implanted. For instance, materials for bone substitution need to be strong enough and can be adapted by joints. In addition, bone has a significant role as the whole body's structural support. Hence, mechanical properties of the biocompatible materials need to be strong enough to be used as implants.

Recently, biocompatible composites are highly used in biomedical applications. One of the composites has potential as the implants especially in bone recovery Hydroxyapatite Mineralized Collagen (HMC) composites. The human body that normally can be substituted and developed by biomimetic composite materials is bone tissue. Hence this paper reviews the HMC composite pertaining to the properties, applications, and manufacturing method for producing this composite. The paper also discusses general composite types, applications of general biomaterials, and the manufacturing process.

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GENERAL BIOCOMPATIBLE COMPOSITE

Composites are artificially manufactured from two or more distinct materials resulting in aggregate and better properties significantly different from its constituent's phases [2][3]. The composites offer flexibility to be used as biocompatible materials. For instant, composites can be modified to resemble bone structure, provide promising scaffold structure as the bone substitution and regeneration. Composites usually have a stiff and strong properties, but they are still light in weight. Generally, composites consist of two-phases which are reinforcement and matrix. For some cases, reinforcement is stiffer, harder, and more robust than matrix [3].

Typically, three types of composites are based on reinforcement: particle-reinforced, fiber-reinforced, and structural composites. Particle-reinforced composites mainly focus on gaining stiffness of the material and increasing its strength and toughness [4]. Hence, it is usually weaker than the fiber composites. However, reinforcement composites are often used because they are less expensive and easy to produce. Fiber composites basically have two types of arrangement, which are continuous (long fiber), discontinuous (short fiber) [5]. In addition, there are two types of short fiber namely random and aligned (Figure 1). Continuous fiber mostly has aligned structures such as woven cloth or helical winding, while discontinuous fiber has either random or aligned structures such as chopped fibers, glass, aramid, and carbon.

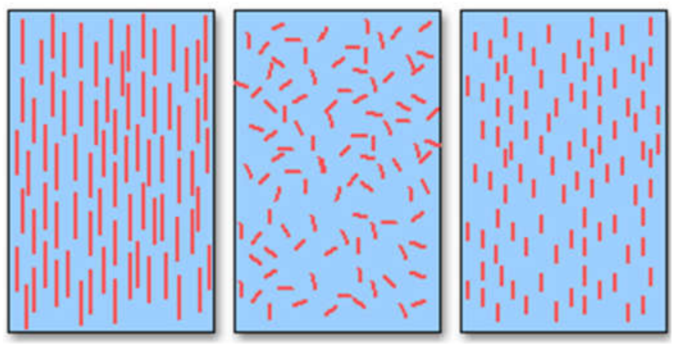

Figure 1. Fibers alignments, long fiber (left), short fiber random (middle), and short fiber aligned (right) [5]

Based on the matrix phase types, composites are divided into three classifications. They are metal matrix composites (MMCs), ceramic matrix composites (CMCs), and polymer matrix composites (PMCs). These three kinds of matrix composites have their own characteristics. Ceramics is brittle but high in strength and stiffness, polymers low in strength and stiffness, and metals are high ductility matrix composite but have intermediate strength and stiffness [3].

Type Of General Biocompatible Composites

There are many developments in biocompatible composite materials. For example, biocompatible Nano Bio glass reinforced poly (ε-caprolactone) composite is developed to boost mechanical and crystalline properties, modulate biodegradation rates, and to imitate tissue composition to obtain nanocomposites that are capable of inducing bone regeneration [6]. This composite is synthesized using in situ ring-opening polymerization method. This material is developed based on poly(ε-caprolactone) or PCL used in biomedical applications combined with NanoBio glass. Another example is intrafibrillar HMC scaffolds. This material is a development of composites materials, especially in tissue engineering. It focused on creating functional scaffolds for bone regeneration [7]. In depth discussion of this HMC composite explains in more detail in section 2.

Application of General Biocompatible Composites

The applications of biocompatible composites depend on expected outcomes. For example, alginate/nano bioactive glass-ceramic (nBGC) composite developed by Srinivasan et al. is useful to regenerate periodontal [8]. The nBGC composite is the mixture of 3% sodium alganite, which dissolved, and 1% nBGC. Figure 2 shows the scheme of alginate/nBGC composite manufacturing.

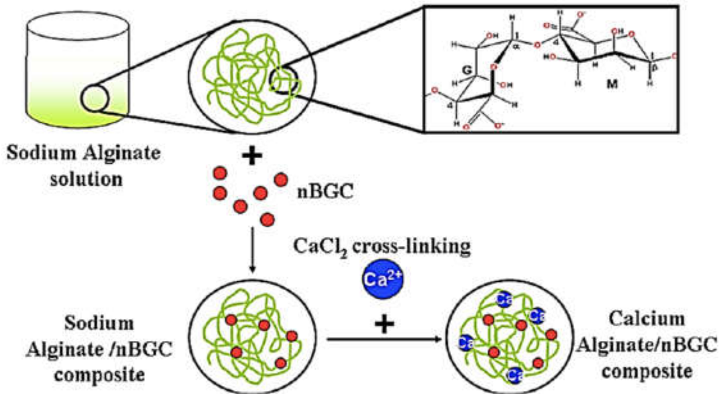

Figure 2. Alginate/nBGC composite manufacturing scheme [8]

The implementation of alginate/nBGC composite as biocompatible materials reduces swelling. Furthermore, alkaline phosphatase activity in human periodontal ligament fibroblasts (HPdLF) cells is increased by the presence of alginate/nBGC composite. Therefore, the presence of alginate/nBGC composite is helpful in periodontal tissue regeneration [8].

Another example of biocompatible composite materials is carbon fiber reinforced polyether-ether-ketone (CFR-PEEK). This composite is appropriate as biocompatible materials for improving the mechanical strength in dental and orthopedic applications [9]. CFR-PEEK manufacturing was conducted using the 3D printing method, which is Fused Deposition Modelling (FDM). During the manufacturing, no toxic was discovered in CFR-PEEK [9]. Therefore, carbon fiber reinforced polyether-ether-ketone can be used in the application of bone repair and regeneration. Some of the applications of this composite are shown in Figure 3.

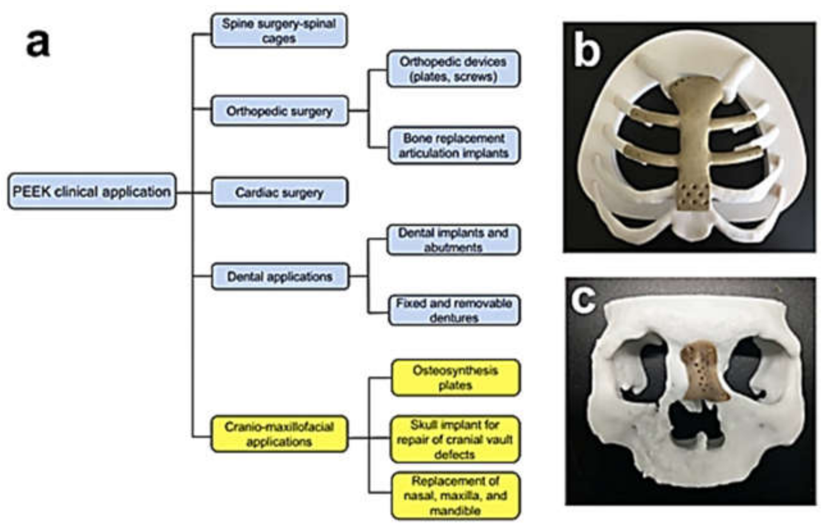

Figure 3. (a) Clinical application of PEEK; application on (b) rib and (c) nasal reconstruction [9]

Manufacturing Process of General Biocompatible Composites

There are various manufacturing processes to generate composite-based biocompatible materials. Following are the manufacturing process according to the type of the matrix composites.

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MMCs Manufacturing Process

One of the examples of MMCs is magnesium (Mg)-based MMCs. The Mg-based MMCs is degradable and suitable for biomedical applications. This composite adopts the concept of bioactive particle reinforced to enhance the mechanical and tribological properties along with the corrosion resistance. In general, the reinforcements for magnesium based MMCs are hydroxyapatite, tricalcium phosphate (TCP), aluminum etc [10][11].

To produce biocompatible MMCs, two manufacturing processes can be applied namely stir casting and powder metallurgy. The casting process can create complex shapes economically over other manufacturing processes. The process is conducted by blending the ceramic reinforced particles with melted matrix metal by mechanical stirring. Subsequently, the molten compound is poured into desired shapes and then solidified. Figure 4 shows the schematic of casting process to create predefined scaffold [12].

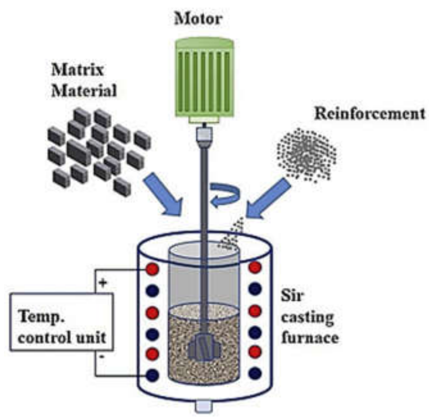

Figure 4. Stir casting illustration [11]

The second process for making MMCs is powder metallurgy. It is a widely used to manufacture MMCs due to its simplicity and cost-effective technique. The process is conducted in three steps: powder blending, green compact composing, and sintering [10]. The power blending is merely mixed the metallic materials with the reinforcement component with considerable compositions. Subsequently, the green compact is prepared by pressing it with a specific force under room temperature. The illustration of the process is shown in Figure 5. The last step is hot press sintering (HP) or sparks plasma sintering (SPS) to sinter the green compact by giving uniaxial pressure and direct current [10][13]. The schematic microstructural of green compact to the final mold can be seen in Figure 6.

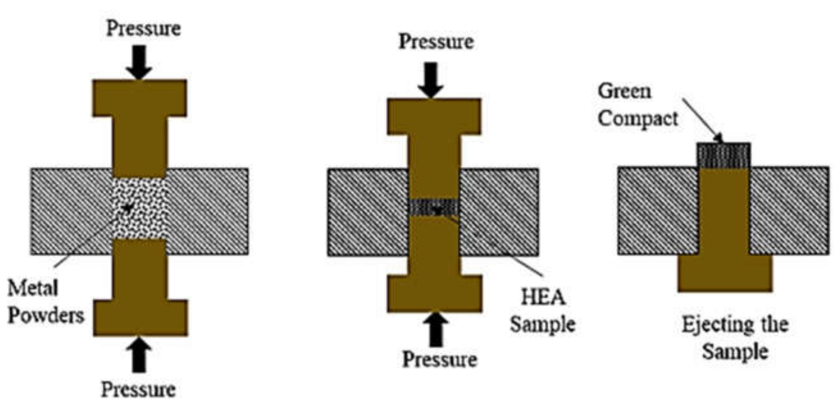

Figure 5. Schematic of green compact preparation [13]

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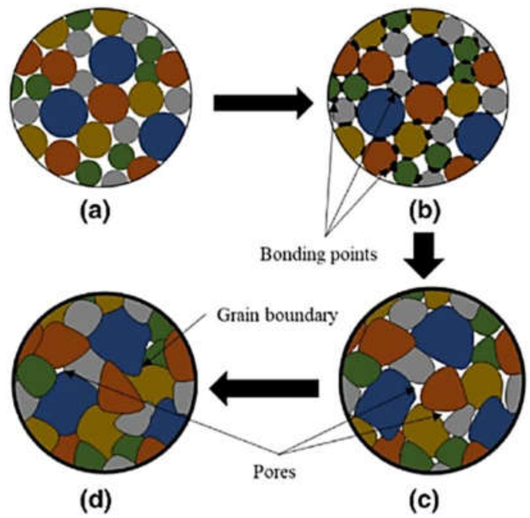

Figure 6. Schematic illustration of sintering microstructure [13]

CMCs Manufacturing Process

In general, ceramic matrix composite (CMC) is widely used in the dentistry field due to its mechanical properties generated from the ceramic based materials such as resistance to high temperature. It has similar properties with a tooth, which are stiff and brittle. One of the ceramic compositions applied as dental ceramic composites is alumina-zirconia-titania. The alumina-zirconia-titania dental ceramic composites is commonly manufactured by powder metallurgy. Firstly, the powders of Al_2O_3 (<50 nm), TiO_2 (<21 nm), and ZrO_2 (12% CeO_2) with purity ≥ 99.5% are prepared and weighed. Subsequently, the powders are dissolved homogeneously in ethanol and dried at 80°C [14]. The mixtures are then molded by uniaxial cold pressing with pressure around 150 MPa to yield green compacts similar to that in Figure 5. The green compacts are sintered in the furnace for 2 hours at 1400°C then molded into the desired scaffold by furnace cooling similar to Figure 6.

PMCs Manufacturing Process

Various polymer matrix composite (PMC) manufacture methods are used for different purposes. In general, there are three steps to produce biocompatible PMCs especially for bone scaffold applications. The steps are synthesizing, manufacturing networks, and yielding the scaffold by 3-D printing. The polymers are synthesized with various techniques, for example, is the synthesis of trimethylene carbonate (TMC) and trimethylolpropane (TMP) using ring-open polymerization (Figure 7) [15]. The polymerization performs above the boiling point of water or around 130°C and under a nitrogen atmosphere. Then hydroxyl-terminated oligomer is dissolved and functionalized with methacrylic anhydride (MAAH) at 25°C temperature. Last, it formed the methacrylate-functionalized oligomer (macromer) in cold ethanol and dried in a 40°C vacuum [15].

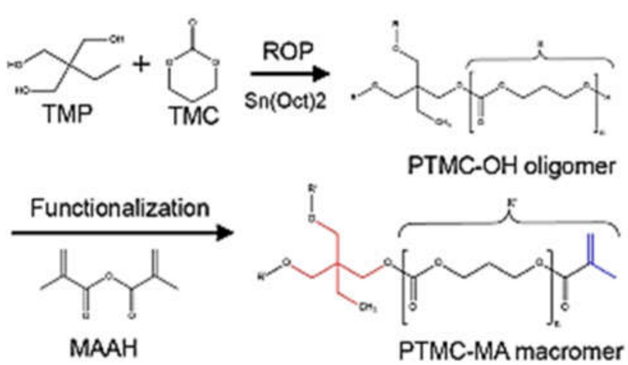

Figure 7. Synthesis of PTMC macromer [15]

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In order to form the bone substitution network, additional steps need to be conducted. The process can be seen in Figure 8. First, resins are fabricated by soaking the poly trimethylene carbonate (PTMC) macromer in propylene carbonate at 70ºC. During the heating, β-tricalcium phosphate powder is blended into the composite resins, and subsequently orasol orange dye is mixed at 25ºC. Then the networks is created by cross-linked and dried at 40ºC vacuum [15]. Last, the scaffold having certain percent of porous as the characteristic of bone is formed using 3-D printing with the prepared resins filament.

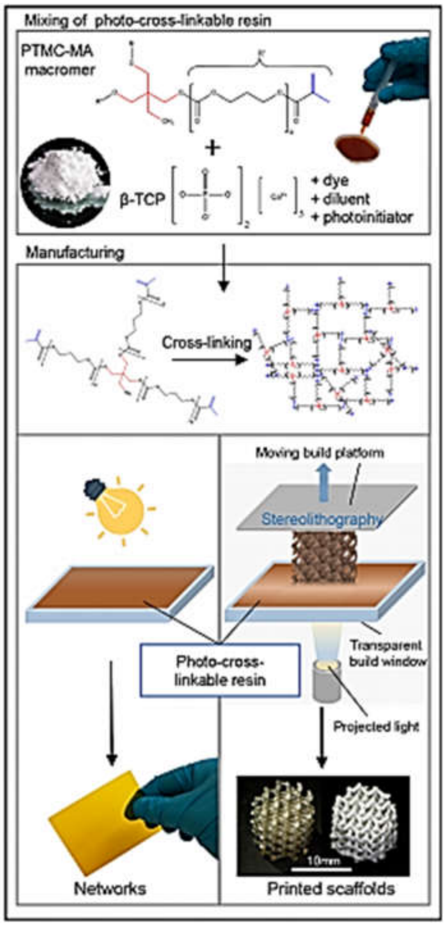

Figure 8. Schematic of manufacturing of the networks and printed scaffolds [15]

BIOCOMPATIBLE HYDROXYAPATITE MINERALIZED COLLAGEN COMPOSITE

The hydroxyapatite and collagen proteins have its composition matching to the natural bone tissue [16]. The combination of both composites can be used in bone regeneration. Many researchers have studied the manufacturing and improving of its applications as implants in the human body. The Hydroxyapatite Mineralized Collagen (HMC) composite can be used to substitute bone as it fulfilled the criteria to be biocompatible materials. To regenerate bone structure, scaffolds are used as an ideal material due to their shape and composition resembled the natural bone. Scaffolds are ideal materials because it defined as a seven-hierarchical-level structure with mineralized collagen fibrils as its second hierarchy level [7]. The Mineralized Collagen (MC) can repair bone defects and regenerate bone tissue.

The HMC composite can be made a porous Mineralized Collagen (pMC) and a compact Mineralized Collagen (cMC). The porous mineralized collagen has pores on the surface, while the compact one has no pores on the surface [17]. The regeneration of bone is different in between pMC and cMC scaffolds. On cMC, the scaffold is not fully replaced by the new bone tissue since it is denser than pMC. As a result, the outline of cMC is visible [17]. In contrast to cMC, pMC underwent biodegradation and entirely replaced by the new bone tissue. Figure 9 shows the difference between pMC and cMC. The pMC had more biodegradation that would be invisible compared to cMC, which is still visible.

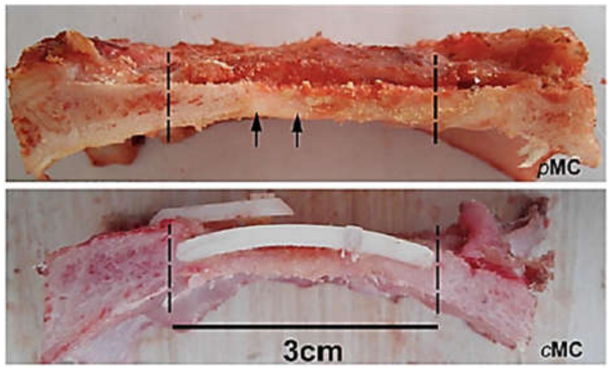

Figure 9. Front view of pMC (top) and cMC (bottom) implantation 3 months after operation [17].

Properties and Characteristics of Hydroxyapatite Mineralized Collagen Composite

The HMC composite is a biocompatible composite-based mixture of hydroxyapatite and collagen. Hydroxyapatite is an abundant protein, and collagen is the essential component in natural bone. Both composites are the most plenteous protein and critical substances in constructing natural bone tissue [19][22]. The hydroxyapatite-collagen (HA-Col) composite has strong osteoconductive properties and high osteogenic gene expression levels inside these mineralized collagen scaffolds. In tissue engineering, the combination of both materials can be used as a biomimetic composite material due to its excellent biocompatibility and biodegradability [7]. To achieve dopant-induced osteogenesis of HA-Col composite, hydroxyapatite components are modified with additional inorganic materials such as gold (Au), carbon (C), copper (Cu), silver (Ag), or manganese (Mn). Manganese, for example, is one of the most essential radioactive elements of the bone. Manganese primarily contributes to body growth and development by contributing to carbohydrate metabolism and mucopolysaccharide synthesis in natural bone [7].

Some mechanical testing such as compressive and tensile tests have been conducted in order to verify the composite mechanical properties the same as the natural bone [16][19][22]. Due to its similarities to the natural bone component, the inorganic HMC can perfectly suit the human bone or even animal tissue for a long time. According to Li et al., three aspects need to be considered to improve the MC scaffold [20]. They are physical, which deals with mechanics and morphology, biological aspects related to the cell and growth factor, and chemical, which includes composition and cross-linking. If these three factors are fulfilled, it can improve the biocompatible scaffold's quality along with its sustainability.

Manufacturing Process of Hydroxyapatite Mineralized Collagen Composite

Collagen as the based composite scaffolds for bone substitution and regeneration can be yielded by three different biomaterials: bio-ceramic, carbon, and polymer [21]. However, this section only focuses on the manufacturing process of the bio-ceramic component, which contains calcium phosphate (CaP), primarily inorganic mineral hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$,]. This mineral construct bone tissue, which illustrates in Figure 10.

Initially, the HMC powder was fabricated. In this step, diluted Hydrochloric Acid (HCl) is dissolved with type I collagen in order to formulate an acidic collagen solution. Then, calcium chloride ($CaCl_2$) and NaH_2PO_4 solution (Ca/P=1.67) were dropped before stirring. Subsequently, it is stabilized the pH up to 7.4 with NaOH solution and gradually blended for 48 hours until the MC gradually formed [17]. After that, the MC is gathered by centrifugation and washed three times with deionized water until the powder is yielded [22].

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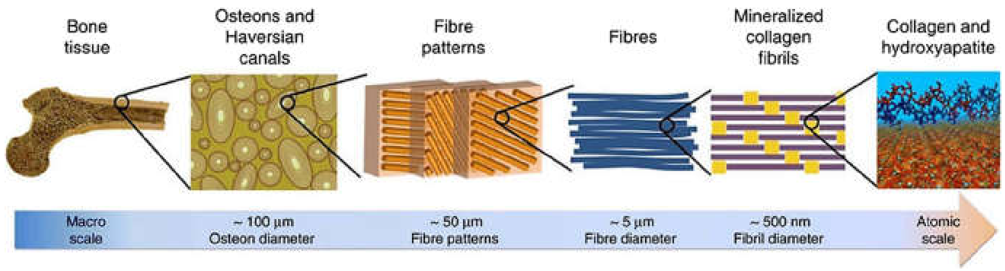

Figure 10. Collagen and hydroxyapatite structure inside the bone tissue [18]

Once the MC powder ready, the scaffold can be manufactured as pMC and cMC. In the pMC, powder was mixed with a biodegradable polymer such as polycaprolactone (PCL), which is dissolved first with 1.4-dioxane to create the porous MC composite scaffold. PCL and MC powder with a proportional ratio is stirred until it forms a homogenous slurry. It is then poured into the selected mold and keep at -20^{o}C for around 12 hours [17]. For sterilization, freeze-drying is needed to detach the organic solvent [22].

In the fabrication of cMC composite scaffold, the MC powder is mixed with 80^{o}C viscous PCL with an even ratio by mechanical force [17]. The mixture then molds to the scaffold's desired shape by cool it down within room temperature [22]. Advance technologies bring the molding part easier and more applicable for any bone structure. Therefore, the existing hybrid process, which collaborates additive manufacturing (AM), which uses 3D printing and CNC machining to smoothen the surface, has significantly assisted bio-machine engineering [23]. The illustration is shown in Figure 11.

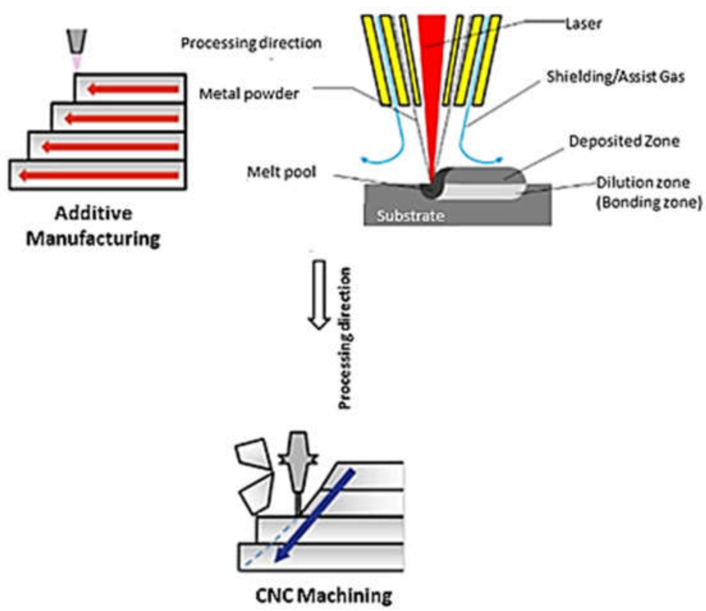

Figure 11. A hybrid process that combines AM and CNC machining [23]

CONCLUSION

Composites become one of the materials that are often used for biocompatible applications. Composites are flexible materials with the characteristics that can be modified depend on the purpose. In tissue engineering, Hydroxyapatite Mineralized Collagen is biocompatible composites material to create scaffold utilized in bone regeneration. The composite implementation is used as a bone substitution, because of its properties and characteristics. The composite is a mixture of hydroxyapatite, a protein to help the bone's growth and mineralized collagen, a mineral that occurred in bone. One of the important manufacturing processes used to create this scaffold, after both important components are mixed and sintered, is a hybrid process. However, further research is still needed to obtain composites that are highly adaptable and acceptable as the scaffolds and bone matrix in bone regeneration process.

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