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Osteocalcin as Bone Formation Biomarker: Literature Review

[1] Almira Hutama, [2] Sonia Natalia Pennsylvania, [3] Elly Munadziroh, [4] Devi Rianti, [5] Titien Hary Agustantina, [6] Yassir Ahmad Azzaim

[1, 2] Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[3, 4, 5, 6] Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

Corresponding Author: **Elly Munadziroh**

Abstract

Background: Tissue engineering represents an integrative approach that combines cells, biomaterials, and biological signaling factors to facilitate tissue regeneration, including the formation of new bone. In the context of bone regeneration, osteocalcin serves as a critical biomarker because it is synthesized during the late stages of osteoblast differentiation and plays an essential role in matrix mineralization and the overall quality of the bone extracellular matrix.

Objective: This statement explains the role of tissue engineering in promoting bone formation and highlights the importance of osteocalcin as an indicator of osteoblast maturation.

Discussion: The success of bone tissue engineering relies on scaffolds capable of supporting the adhesion and differentiation of osteoprogenitor cells. Throughout this process, osteocalcin serves as a key parameter reflecting osteoblastic activity and matrix mineralization. An increased secretion of osteocalcin indicates that the biomaterial or scaffold provides a conducive microenvironment for the formation of new bone tissue.

Conclusion: Tissue engineering plays a significant role in supporting bone regeneration, and osteocalcin serves as a key marker for evaluating the effectiveness of this process. The measurement of osteocalcin provides essential insight into the quality and success of new bone formation within tissue-engineering applications.

Keywords: Osteocalcin, Bone Tissue Engineering, Bone Matrix Mineralization, Human and Health

Introduction

Tissue engineering is a discipline that integrates engineering principles with biological science to reconstruct or replace damaged tissues, either *in vitro* or *in vivo* [1]. This approach is fundamentally built upon three key components: cells, biomaterial-based scaffolds, and biological factors [8]. Scaffolds function as three-dimensional frameworks that are biocompatible, biodegradable, and appropriately porous to support cell adhesion, proliferation, and differentiation throughout the tissue regeneration process [8]. In addition to providing structural support, scaffolds can also serve as carriers for growth factors such as EGF, FGF, VEGF, and TGF-β, which stimulate the formation of new tissues, including bone [1].

In bone tissue engineering, the differentiation of osteoprogenitor cells into osteoblasts can be assessed through the expression of various osteogenic markers. One of the most important of these is osteocalcin, the predominant non-collagenous protein in the bone matrix, synthesized by osteoblasts during the late stages of differentiation [6]. Osteocalcin consists of 49 amino acids and undergoes gamma-carboxylation, enabling strong binding to calcium ions and hydroxyapatite crystals, thereby playing a direct role in bone mineralization [3, 7]. Beyond serving as a marker of osteoblast maturation, osteocalcin contributes to bone quality by regulating the alignment of biological apatite crystals parallel to collagen fibers—an essential determinant of the mechanical strength of bone tissue [7].

Osteocalcin also exhibits endocrine functions, particularly in its decarboxylated form (Glu-osteocalcin), which enhances insulin sensitivity and influences energy metabolism [9]. Given its role as a late-stage maturation marker and a key indicator of matrix mineralization, osteocalcin represents a critical parameter for evaluating the osteoinductive potential of biomaterials used in bone tissue engineering.

Material and Methods

The method used in this article is a literature review with a narrative procedure. The review examines the latest research about osteocalcin secretion with collagen-hydroxyapatite scaffold *in vitro*. To identify the most relevant publications, international databases such as Science Direct, PubMed, ResearchGate, and Google Scholar were used to access secondary data. The selection criteria included full-text availability for studies published between 2020-2024.

Discussion

Tissue Engineering

Tissue engineering has been developed to create scaffold biomaterials capable of integrating with host tissues while enhancing cellular adhesion, proliferation, bioavailability, and differentiation [4]. Bone tissue engineering (BTE) is a complex process that involves the use of cells, engineered materials, and physicochemical factors with the aim of optimizing material design to support the formation and regeneration of new bone [10].

Scaffold design is central to BTE, as implanted scaffolds provide the necessary substrate for cell adhesion, proliferation, migration, and differentiation at the site of bone damage [16]. During bone regeneration, the scaffold not only serves as a surface for cell attachment and growth but also as a structural framework that preserves space for new tissue formation. Porosity and pore interconnectivity are critical features, as they determine the diffusion of nutrients and oxygen as well as the removal of cellular metabolic waste. In addition, scaffolds can function as bioactive delivery systems capable of releasing growth factors in a controlled manner to guide osteogenic differentiation.

The regulation of bone-related cells—including osteoblasts, angioblasts, immune cells, and others—plays a critical role in directing cellular responses and ultimately determining the outcome of bone repair and regeneration. The first component of tissue engineering is the cellular element, particularly progenitor or stem cells that possess proliferative and differentiative potential. In bone tissue engineering, commonly used cells include osteoblasts, mesenchymal stem cells, and pre-osteoblasts such as the 7F2 cell line. These cells have the capacity to migrate, proliferate, and differentiate into mature osteoblasts when exposed to appropriate biological signals. This differentiation capability forms the foundation of new tissue formation. The application of BTE scaffolds aims to accelerate the repair of bone defects [16].

An ideal biomaterial must exhibit osteoconductive properties (providing a scaffold structure for bone regeneration), osteoinductive capabilities (containing factors that induce bone formation), and osteogenic potential (harboring cells that promote bone formation), as well as bone-binding characteristics that enable integration with native bone [15]. In addition, the biomaterial must be biocompatible, biotolerant, and biodegradable [2].

One example is that collagen-based scaffolds can provide a bone-like microenvironment and enhance bone growth and cellular differentiation [4]. Firouzeh *et al*. [4] reported that the combination of amniotic membrane and hydroxyapatite used as a scaffold in bone tissue engineering (BTE) significantly enhances osteogenic differentiation, as demonstrated by the increased expression of various osteogenic markers. The success of tissue engineering is determined not only by the presence of cells, scaffolds, and biological factors, but also by the ability of these components to collectively establish a stable tissue microenvironment. The interaction between biomaterials and cells plays a decisive role in shaping the biological response. For instance, the chemical composition, surface topography, and roughness of a biomaterial can influence cellular adhesion and subsequently guide the differentiation process. At this stage, osteoblastic differentiation can be evaluated by the expression of markers such as ALP (early phase), COL1A1 (matrix formation), and particularly osteocalcin (late phase).

Tissue engineering has emerged as a highly promising strategy for the development of novel biomaterials, as it enables the creation of scaffolds that function not only as structural substitutes but also as biologically active matrices capable of directing new bone formation. Its major advantage lies in its ability to mimic the natural regenerative processes of the body, resulting in tissue that is of higher quality, more biocompatible, and more stable over the long term.

Bone Formation

The socket healing process consists of four phases: hemostasis and coagulation, inflammation, proliferation, and modeling–remodeling. Osteoblastic activity is reported to be highest within the first four weeks of healing. The production of various growth factors that stimulate osteoblasts, along with an increase in osteoblast volume density, begins around day 14 after extraction. The proliferative phase of bone healing typically starts on day 14 and is followed by the bone remodeling phase around day 28 [5, 13, 14].

During the differentiation of ectomesenchymal cells into osteoblasts, RUNX2 and OSX stimulate several bone-related growth factors, including ALP, type I collagen, bone sialoprotein, and fibronectin, which serve as early markers of osteoblast differentiation. As differentiation progresses, RUNX2 and OSX further induce the expression of osteocalcin (OCN), osteopontin (OPN), and osteonectin, which function as late markers secreted during the final stages of osteoblast maturation [4, 12].

Osteocalcin

Osteocalcin is a glycoprotein that binds Ca^{2+} ions, hydroxyapatite, and collagen, and is secreted by osteoblasts during the intermediate and late phases of osteoblast maturation. It plays a crucial role in hydroxyapatite crystal deposition, crystal maturation, and bone matrix mineralization (Utomo *et al*., 2023, p. 1524; [12]. OCN is the most abundant non-collagenous protein in bone tissue and is traditionally regarded as a marker of mature osteoblasts, as its expression is restricted to fully differentiated osteoblasts [12]. Osteocalcin consists of 49 amino acids and belongs to the group of vitamin K–dependent proteins because its gamma-carboxylation requires vitamin K to enable strong binding to calcium ions and hydroxyapatite crystals [7]. This carboxylation process allows osteocalcin to directly regulate bone matrix mineralization, particularly by forming stable interactions between biological apatite crystals and collagen fibers, which contribute to the mechanical strength of bone [7].

Compared with other osteoblastic markers, osteocalcin exhibits unique characteristics as an indicator of osteogenesis. Markers such as alkaline phosphatase (ALP) and type I collagen (COL1A1) are expressed during the

early stages of differentiation, when cells remain in a proliferative phase and have not yet entered the matrix mineralization stage. In contrast, osteocalcin is expressed only when osteoblasts have fully differentiated and are ready to initiate and sustain the mineralization process [6]. Therefore, osteocalcin is regarded as a late differentiation marker that reflects the final stage of the transition from osteoprogenitor cells to mature osteoblasts.

Osteocalcin plays a structural role in the formation of new bone matrix. Its expression is closely associated with the alignment of biological apatite crystals parallel to collagen fibrils—an organization that is essential for both the compressive and tensile strength of bone [7]. This indicates that osteocalcin not only reflects the presence of mature osteoblasts but also the quality of the mineralization produced. In other words, osteocalcin serves not merely as a marker of cellular differentiation but also as an indicator of the quality of bone regeneration supported by a biomaterial.

In the context of tissue engineering and cell culture research, the timing of osteocalcin expression has become a critical reference for evaluating the success of osteogenesis. In 7F2 pre-osteoblasts—one of the most widely used *in vitro* osteoblastic cell models—osteocalcin expression begins around day 7, marking the transition from the proliferative phase to the advanced differentiation phase [11]. At this stage, early mineralization is being prepared, although the bone matrix has not yet formed optimally. Osteocalcin expression then increases markedly by day 14, a critical period indicating active mineralization and mature osteoblastic activity. This stepwise expression pattern, from day 7 to day 14, is widely used as a biomaterial evaluation parameter, demonstrating a scaffold or composite's ability to support osteoblastic differentiation and mineralization.

In vivo, the proliferative phase of bone healing begins around day 14 and is followed by the remodeling phase around day 28. On the 28th day, early osteoblasts begin to be replaced by mature osteoblasts, accompanied by an observable increase in bone density [14]. The final stage of osteogenesis is characterized by progressive mineralization and bone maturation beginning on day 14, with peak secretion of the markers OCN and OPN occurring around day 21 [4, 12].

Conclusion

In conclusion, bone tissue engineering as the main principle of scaffold design is important to create a biomaterial that is capable of integrating with tissue and providing cell adhesion, proliferation, bioavailability and differentiation; thus helping the bone formation process. Bone formation consists of four main phases; hemostasis, coagulation, inflammation, proliferative, and remodelling. Secretion of osteogenic markers start peaking from the proliferating phase at 14th day. Osteocalcin as bone mineralization marker secretion peaked at around day 14 to day 21.

Conflict of Interest

The author declares no conflict of interest

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