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Osteogenic activity of lactoferrin and its application in contemporary dentistry

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ABSTRACT

Introduction: Lactoferrin (Lf) is a protein in the transferrin family with many biological functions. One novel activity of lactoferrin described recently is its regulatory function in bone morphogenesis. Lactoferrin has been shown to promote the growth, development, and differentiation of osteoblasts as well as to decrease osteoclast survival. Lactoferrin receptors (LfRs) mediate the multiple functions of lactoferrin. This review focuses on LfRs associated with bone and the intestines. The best known LfR is small intestine LfR (intelectin), which facilitates iron absorption and iron metabolism in humans. Many data from *in vitro* and *in vivo* studies have indicated that lactoferrin promotes bone formation by increasing the proliferation of osteoblasts and the ability of cells to synthesize and mineralize the bone matrix. Lactoferrin additionally inhibits osteoclastogenesis, reducing the number of osteoclasts and thus bone resorption. Lactoferrin, with its numerous antimicrobial, anti-inflammatory, and also osteogenic properties has found a number of applications in contemporary dentistry, especially in dental surgery, in periodontology, and in pedodontics.

Summary: This review presents *in vivo* and *in vitro* studies demonstrating the osteogenic and anti-inflammatory activity of Lf and its practical application in oral surgery and dentistry.

Keywords: lactoferrin; bone; oral surgery; dentistry.

INTRODUCTION

Lactoferrin (Lf) is a nonheme iron-binding multifunctional protein of the transferrin family that plays a variety of functional roles, showing immunomodulatory, anticancer, antibacterial, and antiviral properties [1, 2, 3, 4]. As a biologically active protein, Lf exerts broad physiological effects on different types of cells, including bone cells [5, 6], via specific receptors. One novel activity of Lf that has been recently demonstrated is its regulatory function in bone morphogenesis and bone homeostasis [7]. Lactoferrin has been shown to promote the growth, development, and differentiation of osteoblasts (OBs) [8] as well as to decrease osteoclast survival [9, 10]. Due to Lf's osteogenic activity, many *in vitro* and *in vivo* studies have been performed to seek innovative methods to create biomaterials with Lf, in order to reduce the local imbalance in bone homeostasis occurring in osteoporosis and other bone diseases. The aim of this study was to present the osteogenic activity of lactoferrin and ways of applying in oral surgery.

LACTOFERRIN RECEPTORS

Lactoferrin receptors (LfR) mediate the multiple functions of Lf. These receptors seem to differ among species, tissues, cell types, and even degree of maturation. Reports have described various cells with LfRs; from the point of view of Lf's function on bone, the most important LfR are those associated with bone and with the intestines. Reverse transcriptase polymerase chain reaction (RT-PCR) studies reveal that LfR are expressed at high levels in the fetal small intestine and at lower levels in human intestinal epithelial cells line (Caco-2 cells). Small intestine LfR, also called intelectin, is the best known of the LfRs, and facilitates iron absorption and iron metabolism in humans [11]. Lönnerdal studied the presence of LfRs in the brush border membrane of infant rhesus monkey intestine and from fetal and infant human intestines. The binding of the receptor is specific to human and monkey Lf; bovine Lf (bLf) did not bind to the receptor. It has also been found that enzymatic deglycosylation does not affect the binding of human Lf to its receptor, so glycan is not needed for receptor recognition [12]. Since Lf is resistant to proteolytic digestion [13] and LfR is present in the intestinal brush border, uptake of Lf from the lumen to the blood is rapid [14].

Lactoferrin may also interact with heparan sulfate proteoglycans (HSPGs), glycosaminoglycans (GAGs), glycoprotein 330 (gp330), low-density lipoprotein receptor (LDLR), nucleolin, and the asialoglycoprotein receptor [15, 16, 17, 18, 19]. In fact, LfR's propensity to bind to most cells confers protection against infection by some viruses [20]. Besides, the low-density receptor-related protein/alpha 2-macroglobulin receptor (LRP/alpha 2 MR) and gp330 both may function as endocytosis-mediating

receptors for a large number of ligands (including Lf) in different organs [19]. Bovine Lf has also been used to treat intestinal giardiasis when internalized by receptor-mediated endocytosis [21]. Of the various LfR, only low-density lipoprotein receptor-related proteins 1 and 2 (LRP1 and LRP2) are present on OBs [22]. The low-density lipoprotein receptor-related proteins 1 is at least partially responsible for LF's mitogenic effect on OBs through activation of LPR1-mediated p42/44 mitogen-activated protein kinase (MAPK) [22]. Surprisingly, induction of osteoblast survival by Lf is not dependent on LPR1 [23]. Hou et al. discovered that Lf induces IGF-1/IGF-1R expression in a concentration-dependent manner, and that it induces proliferation but inhibits apoptosis of OBs through mediation of IGF-1R [24].

Lactoferrin has beneficial effects on bone resorption by reducing the number of osteoclasts formed from precursor cells. Although effects of Lf on osteoclast development have been noted, Lf does not affect mature osteoclast activity [8]. Osteoclasts are unique cells whose hyperactivity is involved in bone pathologies such as osteoporosis and osteolytic diseases. Osteoclasts derive from a mononuclear phagocytic lineage that expresses cluster of differentiation 14 (CD14) on the cell's surface [25]. Factors such as hormones, cytokines, and growth factors that regulate osteoclast differentiation and activity act directly on osteoclasts and their precursors, or indirectly OBs to modify the expression of proteins that control osteoclastogenesis, or use a combination of both mechanisms, direct and indirect [26, 27]. The mechanisms involved in the effect of Lf on osteoclasts are not known, and the receptor responsible for mediation of Lf's effect on osteoclasts has not yet been discovered [28]; however, it may be associated with the indirect influence of Lf on bone metabolism through modulation of cytokine production [29].

IN VITRO STUDIES

Osteogenesis is a complex process that includes the differentiation of mesenchymal cells into preosteoblasts and OBs, leading to the synthesis and deposition of bone matrix proteins [30]. Data obtained from *in vitro* studies support the thesis that Lf has osteogenic activity. To examine the *in vitro* influence of Lf on osteogenesis, an undifferentiated mesenchymal cell line, or one isolated from bone marrow cells, was used. One such cell line are myoblast progenitor cells (C2C12 cells), which originate from undifferentiated mesenchymal cells; they have the capacity to differentiate not only into myoblasts, but also into OBs, chondroblasts, and adipocytes [31]. In experiment by Yagi et al., C2C12 cells were cultured in medium supplemented with Lf in increasing concentrations [32]. The addition of Lf resulted in the proliferation and differentiation of C2C12 cells into osteoblastic and chondroblastic lineages. It was noted that there was an increase in the activity of alkaline phosphate and increases in the expression of Runx2 (essential for OB differentiation and chondrocyte maturation) of Sox 9 (which regulates many developmental events, such as cartilage development), and of osteocalcin (OC), which plays an important role in bone turnover and formation. Muscle cell markers have also undergone reduction after the addition of Lf. Messenger RNA (mRNA) analysis indicated that all effects were exerted by LRP1 LfRs expressed in C2C12 cells [32].

The effect of Lf on osteoblast-like cells has been investigated in animal and human OBs. The study Cornish et al. [8] make use of primary cultures of fetal rat OBs enzymatically digested from 20-day rat calvariae, human OBs from enzyme-treated normal trabecular bones (obtained from patients undergoing knee or hip arthroplasty), and human osteoblast-like cell line (SaOS-2). The cells were cultured in medium supplemented with Lf isolated from fresh bovine skim milk. Additionally, a model of osteoclast function was created utilizing cultures of bone marrow cells obtained from long bones of mice (aged 4–6 weeks) and rat osteoclasts isolated from the long bones of 1-day-old rats cultures. The study showed that bLf stimulated osteoblast proliferation and the differentiation of rat osteoblast-like cells, as well as of the SaOs-2 cell line and human OBs in primary cultures. Lactoferrin also reduces OB apoptosis and has the ability to inhibit osteoclastogenesis in mouse bone marrow cultures, while having no effect on bone resorption by isolated mature OBs. Those researchers concluded that Lf is a potential therapeutic target in some bone disorders [8].

The same proliferative effects of bLf on OBs was observed in the mouse osteoblastic clonal cell line MC3T3-E1 *in vitro*. Supplementation of cultured medium with 100 μg/mL of bLf resulted in the stimulation of OB proliferation, and increased the length of the G2/M and S phases of the cell cycle [33]. It was established that the bLf can induce the proliferation of MC3T3-E1 OB cells by stimulating 3 members of the mitogen-activated protein kinases (MAPK): extracellular-signaling-regulated kinase (ERK), c-Jun N-amino-terminal kinase (JNK), and p38 kinase [34].

Recently, new biomaterials with recombinant human or animal Lf have been sought to promote osteogenic differentiation of cells *in vivo*, so as to maintain bone homeostasis. There have been trials to find such biomaterials by making a culture of mesenchymal stem cells (MSCs) isolated from rabbit bone marrow in the presence of hydroxyapatite (HA) nanocrystals with surface loaded bLf. The hydroxyapatite and Lf have been shown to induce MSC differentiation and enhance anabolic bone activity, and can be expected to be potential tools in bone regeneration. The coupling of HA and Lf has been shown to play a role in the induction of osteogenic markers, represented by the stimulation of alkaline phosphatase (ALP) activity and the expression of osteogenic-associated genes, such as *RUNX2, BMP2, SPARC* [35].

The same *in vitro* model, HA nanocrystals with Lf was used to test the behavior of OB and osteoclast growth. The preosteoblast cell line MC3T3-E1, a model of OBs, and the murine monocyte/macrophage cell line RAW 264.7 Osteoclasts (a model of osteoclastogenesis) were used in the experiment. The cells were cultured separately or cocultured in the presence of HA-Lf. The hydroxyapatite nanocrystals coupled with the bLf exerted a positive effect on the viability of OBs and decreased

the percentage of apoptotic cells. Additionally, after 14 days of culture, HA-Lf significantly upregulated the expression of an osteoblast-specific transcription factor (Osterix) and of integrin-binding sialoprotein (IBSP) genes. The HA-Lf system reduced osteoclast formation and the expression of Integrin (Itgβ3). The crosstalk between OBs and osteoclasts has been evaluated in coculture. The HA-Lf and Lf induced a significant down-regulation in the expression of the osteoclast-associated immunoglobulin-like receptor (Oscar) and *Catepsin K* (*CtsK*) genes. The results reveal that HA and Lf act in synergy when coupled together as an anabolic factor for OB and bone matrix deposition, and as an inhibitor of osteoclast formation and activity. The authors suggested that the HA-Lf system could be used for several bone-related pathologies, and in future in tissue engineering and regenerative medicine [36].

A similar experimental model was created by Vandrovcova et al. to evaluate the effects of Lf in prepared collagen hydrogels. An artificial extracellular matrix (aECM), consisting of collagen type I fibrils, was formed in the presence of Lf at two different concentrations (0.5 and 1 mg mL-1). In this model, the effects of aECM on adhesion, growth, and osteogenic differentiation of human osteoblast-like Saos-2 cells were investigated. The greatest numbers of cells were found in cultures with collagen-Lf coating (particularly with higher concentrations of Lf), which produced higher levels of osteocalcin. These authors concluded that Lf promotes adhesion, growth, and osteogenic differentiation of Saos-2 cells, and could be used as a component of bone implant coating [37].

Another promising model was made with the use of the type-1 collagen gel used as a culture system in which OBs were grown in a 3D structure, affecting cell shape, cell-cell interaction, and response to soluble factors [38, 39, 40]. In the study by Matthews et al. (2014), primary calvarial OBs isolated from male and female rats and murine preosteoblastic MC3T3-E1 cells were cultured in 3D type-1 collagen gels with the medium supplemented with growth factors inducing osteoblast proliferation (bLf, TGF-β, and PDGF) [40]. Increasing concentrations of lactoferrin induced a dose-dependent increase in the proliferation of cells. Cell differentiation was determined by mineral staining and osteocalcin, bone sialoprotein, alkaline phosphatase, and dentin matrix protein gene expression analysis. The cells grown in 3D gels showed positive mineral staining and the induction of OB marker genes earlier than did cells grown in a 2D culture (on plastic) [40]. On the other hand, the effect of bLf on OB-induced extracellular matrix (ECM) calcification was also studied [41] with human osteosarcoma-derived MG63 cells as a model. The cells were cultured in plates coated with type I collagen in medium supplemented with bLf. The culture of the cells under osteogenic conditions resulted in significant stimulation of ECM calcification, accompanied by significant promotion of osteocalcin production in the late stage of the osteogenic process (week 3), and in a significantly elevation of ALP activity. The results obtained, with the influence of bLf on MG63 cells, suggest that a type I collagen membrane is useful as a drug delivery carrier for Lf in bone tissue engineering [42]. As was shown in a biochemical and biophysical studies [43], recombinant human Lf developed from brown rice grains [44] was similar to native human Lf (nhLf). Primary rat OBs (using the procedure of Cornish et al. [8]), cultured with the supplementation of holo-rhLf in increasing concentrations, showed a significant increase in cell growth at every concentration of rhLf [43].

Lactoferrin is a component of human secretions, including saliva [1, 4, 45]; it is postulated to favor repair mechanisms by inducing cell differentiation [46]. For this reason, its time-dependent influence on osteoblast-like cell morphology and proliferation capacity was assessed. Murine MC3T3 OBs were cultured in a medium containing human saliva collected from healthy volunteers through stimulation. The presence of saliva in the medium significantly reduced the proliferation capacity of MC3T3 OBs and resulted in the downregulation of key marker genes of OB differentiation, such as RUNX2, osteocalcin, ALP, alpha-1 collagen (Colla1). However, the cultured media contained active salivary enzymes (alpha-amylase, lysozyme, peroxidase, and collagenase), which harm OB-like cells. It was therefore recommended that the clinical contact period of saliva and bone should be shortened as much as possible [47].

IN VIVO STUDIES

In vivo experiments have also been performed to confirm the osteogenic function of Lf documented in the *in vitro* studies. In a murine model, the effects of bLf were tested with increasing doses of Lf, given as daily injections over the periosteum of the right hemicalvaria in normal adult mice for 5 days. The bLf produced increased new bone formation [8]. No positive effect of Lf on the amount of new bone formation was observed in experimentally induced bone defects in rabbit calvaria [48, 49]. Histological and histomorphological analyses indicated that sites filled with and without the addition of bLf were associated with the formation of a mixture of woven and lamellar bone, although the differences between the groups were not statistically significant with regard to the amount of new bone formation [49]. The authors concluded that although there was no significant difference between the tested groups, it seemed that the addition of LF increased bone formation [48, 49].

The properties of Lf in the regeneration of bone have been confirmed in a similar study performed in adult male Fischer rats [50]. Non-critical-sized calvarial bone defects (2.7 mm) were trephined into the dorsal bone of both sides of a mid-sagittal suture. An absorbable collagen sponge was placed in the bone defects. After that, the animals were treated with Lf injected intraperitoneally. Histological and histomorphometric examination after 4 weeks showed that, in both Lf-treated animals, new bone was formed and OB-like cells were observed around the bony rims. In the control animals, the absorbable collagen structure had been absorbed, and the defects had filled with dense fibrous connective tissue, with minimal new bone formation. Additionally, there were more OB-like cells in the Lf-treated groups than in the control group [50].

To assess the effect of Lf on bone regeneration during distraction osteogenesis, an experiment was performed using unilateral osteodistraction in the right tibia of male rabbits. The experimental group of rabbits was treated with bLf (85 mg/kg/day) orally from the commencement of the distraction until the end of the experiment. The results from radiological and histological examinations (4 and 8 weeks of treatment) showed that bLf treatment resulted in earlier bone mineralization and better new bone formation in the distracted callus. In the serum of the rabbits, the level of bone alkaline phosphatase (BALP) was higher. The results also showed the increased expression of osteoprotegerin (OPG) mRNA (OPG inhibits osteoclast differentiation and suppresses osteoclast bone resorbing capabilities) and a decreased level of receptor activator for nuclear factor kappa-B ligand (RANKL) mRNA in the distracted calluses. Immunohistochemical examination showed an increase in the number of OPG-positive cells without any difference in the number of RANKL-positive cells in the calluses. These results pointed to a decrease in the RANKL/OPG ratio (the key regulatory system of bone resorption). The authors suggested that treatment with bLf could promote bone regeneration during distraction osteogenesis in rabbits, and that the OPG/RANKL/RANK system might be a major mechanism for increased bone formation and decrease bone resorption [51].

The osteogenic activity of bLf was also confirmed in our study (unpublished data), in which new bone formation in experimentally induced defects in a rabbit frontal bone was observed after a month. Bovine Lf was introduced orally in the form of an aqueous solution of freeze-dried colostrum (bovinum Colostrigen Genactiv, Poland) at 100 mg/kg bw/day (Fig. 1).

A similar mechanism for improving bone mass and microstructure using bLf was observed in an ovariectomized (ovx) rat model [52]. The ovx 6-month-old virgin rats were treated with increasing doses of bLf for 6x months. One group of ovx rats was supplemented with E2 as a positive control. After the treatment, the femur and the L2–4 vertebrae were analyzed. Ovariectomized significantly reduced bone and tissue volume, trabecular number and thickness, and increased trabecular separation in both the femur and vertebrae. Treatment with bLf or estradiol protected the bone from ovx-induced effects. The bone mineral density (BMD) volumes increased in the bone of rats treated with higher doses of bLf, while lower doses of bLf did not significantly alter BMD. Higher doses of Lf affected the turnover of serum biochemical markers of the bone through significantly increasing levels of osteocalcin and BALP, and decreasing levels of C-telopeptide of the beta 1 chain of type-1 collagen of bone (B-CTx) and of collagen type-1 N-telopeptide (NTX). Additionally, the expression level of RANKL mRNA in the proximal femur was decreased, but the expression of OPG mRNA increased after treatment with higher doses of Lf. The RANKL:OPG mRNA ratio in the bone showed a significant decrease in animals treated with Lf in higher doses, while no changes were observed at the lowest dose of Lf [52].

The effects of Lf on bone physiology have already been studied in an osteopenic animal model. Ovariectomized rats were treated orally with bLf in increasing doses for 3 months. Treatment with bLf protected against reduction in bone volume, trabecular number and thickness, and an increase in trabecular separation. Treatment with Lf also resulted in substantial protection against loss of bone mass density and increased mechanical strength. Analysis of biochemical markers of bone remodeling (serum Ca and osteocalcin) indicated greater bone formation and reduced bone resorption. Bovine Lf in higher doses also led to a significant decrease in calcium and significantly elevated the level of serum calcitonin, while suppressing production of serum tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) [53].

Blais et al. used ovx C3H mice and sham-operated mice in an *in vivo* animal model of postmenopausal osteoporosis. It was shown that supplementing the control diet with bLf improved bone mineral density and femoral failure load in a dose-dependent manner [9]. The improvement in bone status caused by bLf could have been mediated by modulation of the immune function, including by preventing lymphocyte activation and releasing cytokines in the bone microenvironment [54]. Research using animal models of postmenopausal osteoporosis has

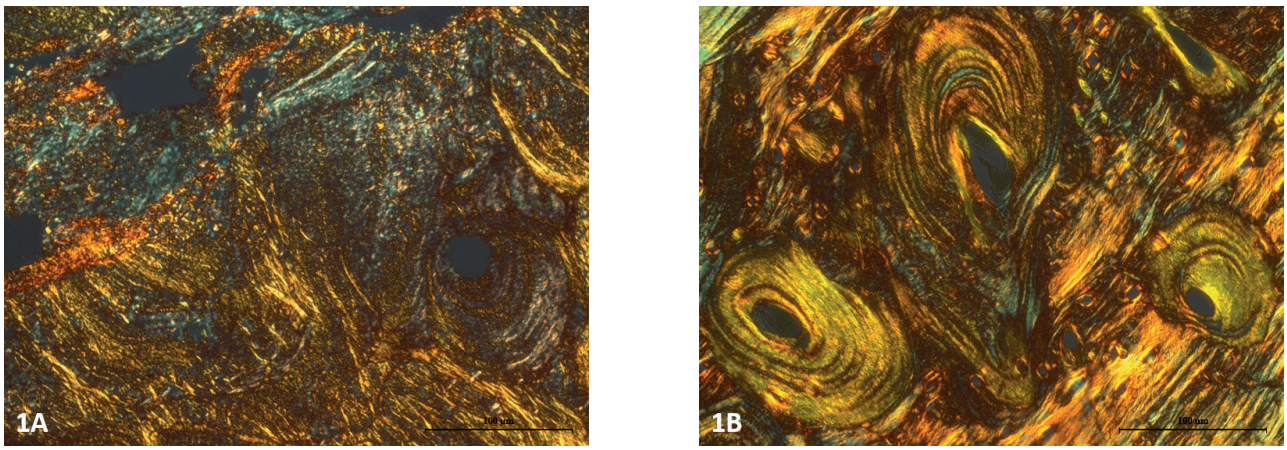

FIGURE 1. Bone sections stained with picrosirius red and imaged with polarized light. Organization of collagen fibers in the frontal bone of control (A) and experimental (B) rabbits treated with bLf, one month after an experimentally induced defect. The organization of the fiber appears less orderly, with abundant thin (yellow–greenish) collagen fibers in the control (A) and collagen fibers following osteonal organization with abundant yellow collagen fibers in the experimental specimens (B). Objective magnification × 40

clearly indicated that supplementation with Lf can suppress osteoclast-mediated bone resorption and prevent bone loss. Lactoferin thus appears to be a promising candidate for the development of an anabolic therapeutic factor for osteoporosis [28].

As mentioned above, Lf appears to be a promising candidate as a therapeutic factor for osteoporosis [28]. Bharadway et al. investigated the benefits of supplementing with milk RNAse-enriched Lf (R-ELf) and calcium on the bone health of postmenopausal women, in comparison to a control group receiving only the calcium supplement. R-ELf significantly reduced bone resorption markers and simultaneously increased bone formation markers without any significant side effects. Additionally, R-ELf supplementation could achieve significant change in bone turnover markers within a short period, within only 3 months of supplementation [55]. More evidence of the beneficial role of Lf on bone health comes from experiments in postmenopausal animal models. Dietary Lf supplementation preserves bone mass and microarchitecture in ovariectomized rats, improving bone strength and bone mineral density [53]. Another study showed that oral bLf supplementation improved bone mineral density and femoral failure load of ovx mice in a dose-dependent manner [9].

Data obtained from *in vivo* studies support the osteogenic function of Lf and its practical application.

APPLICATION OF LACTOFERRIN IN ORAL SURGERY AND DENTISTRY

Lactoferrin, a protein with numerous antimicrobial, anti-inflammatory, and osteogenic activity properties, has a range of applications in contemporary dentistry [4], including in disciplines such as dental surgery, periodontology, and pedodontics. Implantoprosthetics combines interdisciplinary knowledge in dental surgery and prosthodontics; the increasing frequency of implant therapy is associated with great benefits of this treatment for patients with missing teeth, providing stable support for prosthetic works and contributing to restoring stomatognathic system function, improving aesthetics, and eliminating the discomfort associated with removable prostheses. However, patients who qualify for implant therapy often lack the appropriate amount or quality of bone tissue in the site of the planned treatment.

Some dental implant procedures involve the application of short dental implants, bicortical implants, although the majority of patients prefer the procedure known as "two-stage placement", with the regeneration of lost bone tissue or enough bone density to hold the implant in place [56]. In first stage of the procedure, a bone graft is required, and autogenous grafts are the gold standard in bone regeneration [57]. Bone grafting can be done either before or during the implant procedure. It is not always possible to obtain an adequate amount of bone tissue from the patient during the autogenous graft, so xenomaterials are often used as an alternative [58]. In order to increase regenerative properties, the materials are linked with anabolic factors [59] such as Lf, which has documented anabolic properties towards bone tissue. In a study by Takaoka et al., the use of a gelatine carrier for Lf enabled a prolonged release of the glycoprotein to increase the activity of the osteoblasts and improve local bone regeneration [60]. However, in the study of Paknejad et al., Lf in combination with anorganic bovine bone (ABB) was inserted into bone cavities but no statistically significant differences were observed between ABB alone and LF+ABB in the bone regeneration [49]. Another study investigating the effect of Lf on bone resorption of a midpalatal suture during rapid expansion in rats showed that the bone:tissue volume ratio and the bone mineral density of the suture bone were significantly higher in expansion animals treated with Lf than in the expansion group without Lf. Moreover, the activity of OB-like cells and the amount of new bone formation were stimulated in the group treated with Lf, while the activity of the osteoclasts showed no significant differences between groups. These findings reveal that Lf can stimulate bone volume and bone density in midpalatal sutures during the suture remodeling process under tensile force, but that this effect is not caused by the reduction in bone resorption [61].

A gelatine microsphere (GM) carrier with bLf (GM-bLf) in combination with ABB was used in 1 study to regenerate bone tissue defects formed around dental implants in pigs [62]. This provided new bone formation at a higher percentage, as well as faster regeneration than in a group where only inorganic bovine material was implanted. Systemic Lf administration in bone defect regeneration conducted on animal models led to new bone growth in micro-CT images 2 weeks after the surgical procedure [50]. Oral administration of bLf was also demonstrated to have an advantageous effect on bone formation after the process of osteodistraction [51].

The use of Lf in implantology is also associated with its antiresorptive properties for titanium. Implant abutments are usually made of titanium alloy and are in contact with oral mucosa. An antimicrobial barrier should be created between the implant and the mucosa. This hermetic contact helps avoid the occurrence of peri-implantitis. Implant abutments covered with an Lf layer inhibited bacterial adhesion and showed antimicrobial activity. The use of Lf as a covering layer for titanium alloy elements is effective in avoiding peri-implantitis [63, 64].

According to Ishikado et al., systemic administration of liposomal bLf (L bLf) inhibits the production of the inflammatory cytokines TNF-α, IL-1β, and IL-6 in patients with periodontal disease. Liposomal bLf supplementation can therefore serve as an additional method of treatment for periodontitis [65]. Moreover, the measurements of Lf and α1-antitrypsin in gingival retention fluid can be used in the diagnosis of periodontal disease [66].

There is evidence that bLf naturally inhibits the adherence of Streptococcus mutans to enamel HA. Bovine Lf inhibits saliva-induced Streptococcus mutans aggregation. This action is due to the amino acid of Lf (Lf411). S. mutans is the main bacteria which causes tooth caries. A study by Oho et al. showed that the Lf domain that binds to salivary film lies in residues 473 to 538. This region is also hidden by a disulfide bond formation

between Cys481 and Cys532 in the Lf411 fragment. This part of Lf strongly inhibits the adhesion of S. mutans to saliva-covered HA beads ($p < 0.05$). It was also reported that shorter fragments did not show significant adherence. Bovine Lf contains the antimicrobial domains lactoferricin B (Lfcin B) and lactoferrampin (Lfampin), which can exterminate bacteria and fungi, including *C. albicans* and *E. coli* [67].

Lactoferrin is also used as an ingredient in toothpastes which are intended for patients diagnosed with severe early childhood caries. It has been shown that the use of tooth paste with Lf, lysozyme, and lactoperoxidase significantly reduces the level of S. mutans and L. acidophilus in the saliva. A reduced level of microorganisms avoids disease progression [68]. Due to its multipotential properties, Lf can be successfully used in a variety of dental specialties.

CONCLUSION

Lactoferrin is a natural host protein with many biological functions. Lactoferrin promotes proliferation, growth, and the development of OBs. It also stimulates bone regeneration by inhibiting osteoclastogenesis and through antiapoptotic action. Moreover, Lf has antimicrobial and anti-inflammatory activity. All of these reasons mean that Lf has many applications in oral surgery.

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