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Local administration of calcitriol positively influences bone remodeling and maturation during restoration of mandibular bone defects in rats

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ABSTRACT

The aim of this study was to investigate the influence of calcitriol on osteoinduction following local administration into mandibular bone defects. Calcitriol-loaded absorbable collagen membrane scaffolds were prepared using the polydopamine coating method and characterized by scanning electron microscopy. Composite scaffolds were implanted into rat mandibular bone defects in the following groups: no graft material (control), bare collagen membrane (CM group), collagen membrane bearing polydopamine coating (DOP/CM group), and collagen membrane bearing polydopamine coating absorbed with calcitriol (CAL/DOP/CM group). At 1, 2, 4 and 8 weeks post-surgery, the osteogenic potential of calcitriol was examined by histological and immunohistochemical methods. Following *in vivo* implantation, calcitriol-loaded composite scaffolds underwent rapid degradation with pronounced replacement by new bone and induced reunion of the bone marrow cavity. Calcitriol showed strong potential in inhibiting osteoclastogenesis and promotion of osteogenic differentiation at weeks 1, and 2. Furthermore, statistical analysis revealed that the newly formed bone volume in the CAL/DOP/CM group was significantly higher than other groups at weeks 1, and 2. At weeks 4, and 8, the CAL/DOP/CM group showed more mineralized bone and uniform collagen structure. These data suggest that local administration of calcitriol is promising in promoting osteogenesis and mineralization for restoration of mandibular bone defects.

1. Introduction

Achieving satisfactory bone regeneration *in vivo* remains an important goal in orthopedic and dental clinical applications. Growth-factor-based tissue engineering technologies have been widely studied, with much of this research focused on bone morphogenetic proteins (BMPs), which have favorable osteogenic potential [19,35,48,52,56]. Despite that, their use is going to be limited due to several drawbacks including their rapid degradation, high costs, safety and efficacy concerns, osteolysis, ectopic bone formation, and soft tissue swelling [1, 15,41]. Recently, it was reported that some drugs and bioactive substances which have stable and strong activity, low costs and high biological safety could also be used to regulate tissue growth [23,27]. Calcitriol, also called 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D3$), is the active form of vitamin D3 and is involved in various physiological processes, including calcium homeostasis, bone metabolism and immune response [22]. It functions by binding to a single vitamin D receptor (VDR), which is a member of the nuclear hormone receptor superfamily [16]. Vitamin D-deficiency causes impairment of bone mineralization, resulting in rickets in infants and osteomalacia in adults [50]. Moreover, mice lacking VDR exhibit impaired bone formation [62]. Conversely, the administration of vitamin D to ovariectomized or rachitic animals can relieve impaired bone mineralization [47]. In addition, clinical studies show that vitamin D treatment can reduce bone fracture risk in age-related bone loss, postmenopausal osteoporosis and drug-induced osteoporosis [2,11,38]. Besides the overall positive effects on bone, calcitriol has also been shown to regulate transcription of the collagen gene in osteoblasts [20] and is a potent transcriptional activator of genes encoding alkaline phosphatase (ALP), osteocalcin (OCN) and osteopontin (OPN) [39]. Moreover, osteoblasts have shown increased activity after $1,25(OH)_2D_3$ treatment *in vitro* [4].

Based on these studies, we aimed to investigate local administration of calcitriol for the restoration of mandibular bone defects in rats. However, the lack of a suitable local delivery system has previously impeded *in vivo* application of calcitriol. An appropriate scaffold with good biocompatibility and biodegradability is therefore required. More importantly, an

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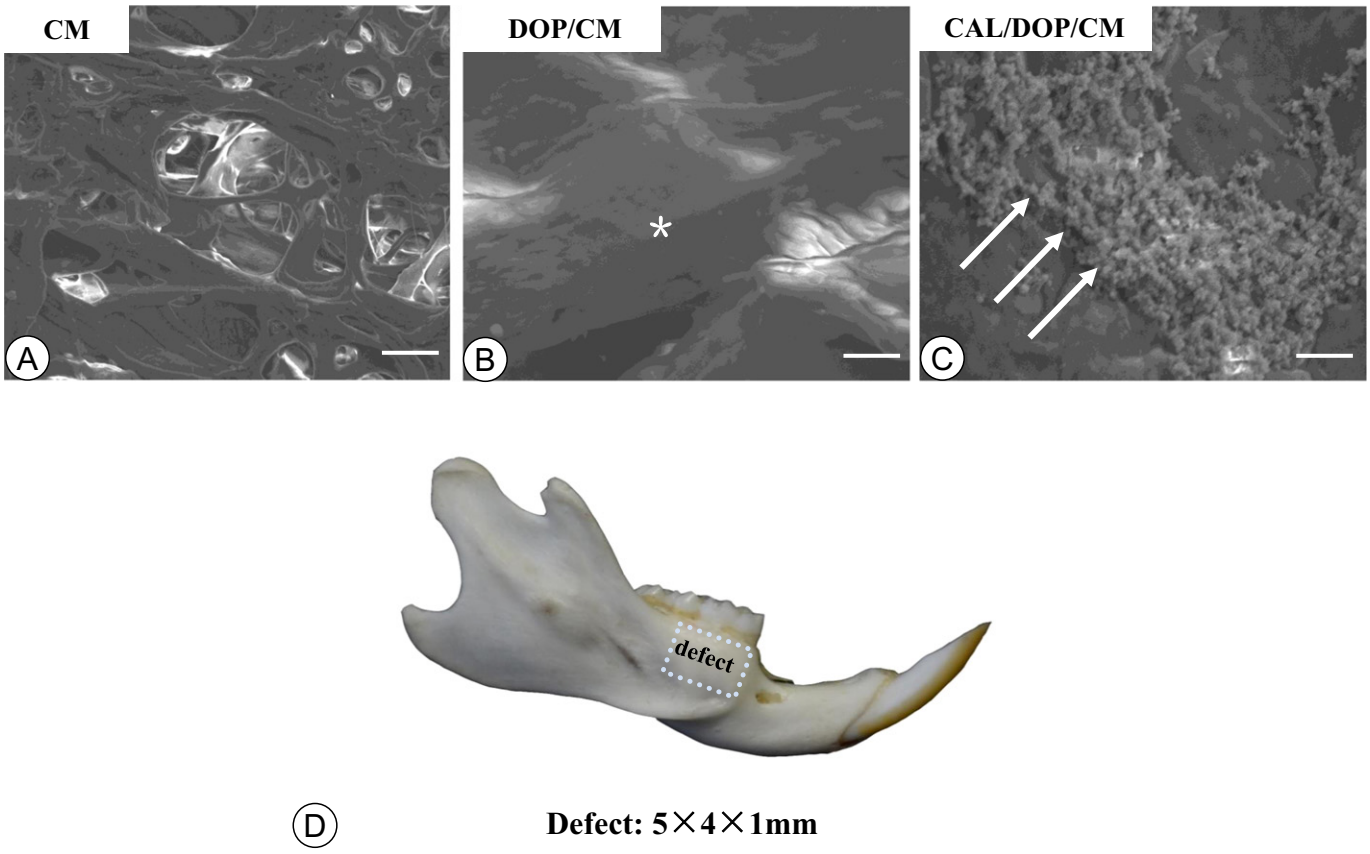

Fig. 1. SEM micrographs and diagram representing mandibular bone defect. SEM micrographs of the original collagen membrane (A), polydopamine-modified membrane (B) and calcitriol–polydopamine composite membrane (C). The diagram represents the surgical window in the buccal surface of the first molar (D). The surface of the original collagen membrane was highly porous with good pore interconnectivity (A). The polydopamine-coated collagen membrane displayed coverage of the polydopamine layer over the membrane surface (asterisk, B). Calcitriol granules were observed distributed on the surface of the scaffolds (white arrows, C). Bar, A–C: 1 μm.

optimal amount of calcitriol to generate osteoinduction needs to be released into the target site over a prolonged period of time. Absorbable collagen membrane, a well-documented barrier membrane in guided bone regeneration (GBR) [7,18], has been used as a growth-factor-combined scaffold. Type-I collagen, as the main component of collagen membrane, is a natural and ubiquitous protein. Its inherent properties include cell attachment and bioactivity, natural biodegradability, amenability to chemical modifications, mechanical cross-linking and self-assembly into a fibrillar gel under physiological conditions, making collagen advantageous for tissue engineering and regenerative medicine applications [13]. In addition, it also showed significant strength and rigidity which can be attributed to its three polypeptide chains structure [13]. Numerous animal studies have shown that collagen membrane is crucially important in retaining BMP levels and ensuring maintenance of space for new bone formation [26]. Moreover, it is reported that loading of collagen membrane with rhBMP-2 shows favorable bone regenerative ability [25]. In view of these results, we selected collagen membrane as the scaffold for loading calcitriol. However, to achieve enhanced immobilization of calcitriol, this carrier material requires additional processing.

Various techniques are available for immobilizing biomolecules onto scaffolds, including gamma ray irradiation and plasma treatment [9,36,54,58]. However, these methods have limitations in terms of penetration depth and possible cleavage of polymeric chains [40]. Recently, a versatile surface modification method, by simply dip-coating with dopamine solution, was reported, and can be applied to almost all solid materials from metals to synthetic polymers [28]. Dopamine can undergo oxidative polymerization and form a stable layer adherent to the surface of biomaterials under basic conditions. Previous studies have applied the polydopamine-coated method to immobilizing animated methoxy-polyethylene glycol, trypsin, bovine serum albumin (BSA), and antibodies [32]. In addition, it is reported that growth factors, such as vascular endothelial growth factor and BMP-2, immobilized

Table 1
Experimental groups.

Groups	Abbreviation	Collagen membrane	Poly-dopamine	Calcitriol
		Absence (−) / Presence (+)	Absence (−) / Presence (+)	Absence (−) / Presence (+)
No graft material	Control	−	−	−
Bare collagen membrane	CM	+	−	−
CM bearing poly-dopamine coating	DOP/CM	+	+	−
CM bearing poly-dopamine coating absorbed with calcitriol	CAL/DOP/CM	+	+	+