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ORIGINAL ARTICLE

Free vascularized medial femoral condyle periosteal flaps in the ankle and foot region: A narrative review

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ABSTRACT

Objectives: The objective of this study was to determine the role and reliability of the free medial femoral condyle (MFC) flap (MFCF) in demanding foot and ankle reconstruction procedures.

Materials and methods: A search of the MEDLINE, PubMed, and Embase electronic databases was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines between January 2008 and September 2023. Articles concerning free MFC bone flaps for reconstruction of the foot and ankle regions were included. Outcomes of interest included flap failure, complications, union rate, time to union, and functional scores.

Results: Twenty studies involving 131 patients met the inclusion criteria. The most common clinical indications for the free MFCF were nonunion, avascular necrosis, and osteomyelitis. The most common sites of nonunion were tibiotalar arthrodesis (50%) and subtalar arthrodesis (33%). Overall, the bony union rate was 93.1%, with a mean time to union of 14.6±0.1 weeks. There were no flap failures reported. Postoperative complications were observed in 39 (29.7%) cases (e.g., delayed donor site wound healing, flap debulking, medial condyle osteonecrosis, and donor site numbness), with 21 (16%) patients requiring further operative intervention. No major donor or recipient site morbidity occurred, except for one case.

Conclusion: Free MFCFs offer a versatile and dependable choice for cases of foot and ankle reconstruction, displaying favorable rates of bone fusion and acceptable complication rates. Existing literature indicates that MFC reconstruction in the foot and ankle is not associated with significant morbidity at the donor or recipient sites. The pooled data demonstrated a 93% success rate in achieving bone fusion in the foot and ankle region, supporting the view that it can be considered another option of treatment.

Keywords: Bony union, foot and ankle bone defects, foot and ankle reconstruction, vascularized medial femoral condyle.

Fracture nonunion, bone defects, joint arthrodesis, and bone avascular necrosis are among the clinical conditions that require bone grafting as an essential component of the treatment.[1,2] Different materials are available to generate an osteogenic response for bone repair, including bone substitutes, growth factors (bone morphogenetic proteins and platelet-rich plasma), biological active membranes (Masquelet), allografts, xenografts, and mesenchymal stem cells, amongst others.[3,4] Autologous bone grafts, however, continue to be the favored grafting method due to their amenable osteoconductive and osteoinductive properties, cellularity, and absence of risks for disease transmission and allergic reactions.[5] On the other hand, their limited availability and associated donor site morbidity constitute significant drawbacks.[6]

There are different types of autologous bone grafts, including cancellous, cortical, corticocancellous, vascularized, and nonvascularized. The most commonly utilized

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anatomical sites for harvesting are the pelvis, distal femur, proximal tibia, and fibula.[5,7]

The medial femoral condyle flap (MFCF) is a common vascularized autologous bone graft that can be used for a variety of reconstruction procedures.[8,9] Thanks to its straight forward harvesting process and low donor site morbidity, the MFCF has gained popularity over the past 20 years in the management of soft and hard tissue defects.[10] When it comes to bony reconstruction, MFCF has been used for the treatment of nonunions, bone defects, osteomyelitis, and avascular necrosis.[11-13] In a meta-analysis of long bone nonunions, Weir et al.[9] reported an overall success rate of 99%. In another study evaluating its effectiveness in scaphoid nonunion, the success rate was 94%.[14] While systematic reviews have been published on the outcomes of treatment for long bone nonunion,[3,9] no systematic review of the literature has been performed on the indications of use and outcomes of the MFCF for the management of foot and ankle procedures. Therefore, this study aimed to evaluate the treatment results of MFCF in foot and ankle reconstructive procedures.

MATERIALS AND METHODS

This narrative review was carried out according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[15] A search was conducted within the databases of MEDLINE, PubMed, and Embase between January 2008 and September 2023 using the following MeSH words: "periosteal flap," "vascularized periosteal flap," "ankle," "foot," "nonunion," "avascular," "necrosis," "osteochondral lesion," "nonunited fracture." The inclusion criteria were articles in English concerning foot and ankle reconstruction with the use of periosteal flaps in adult patients for nonunion, arthrodesis, avascular necrosis, and bone defects. Articles with pediatric patients or animal studies were excluded, as well as reports pertaining exclusively to other anatomical regions (e.g., scaphoid, long bone, and humerus) or articles with bone grafts alone without mentioning periosteal flaps. Cadaveric/experimental, biomechanical, and donor site morbidity studies, as well as manuscripts such as letters to the editor and other technique-related articles, were excluded. The titles and abstracts were screened for relevance, and bibliographies were scrutinized for additional articles.

From each article, data were extracted with regard to study population, study design, patient demographics (age, sex, and body mass index), indication for surgery, site of intervention, number of previous surgeries, operative technique (anastomosis), defect size, time of follow up, healing time, incidence of reinterventions, donor site morbidity associated with medial femoral condyle

TABLE I
Localizations of disease and indication of use

Localization	n	%
Pilon	29	22
Talus avascular necrosis (AVN)	15	11
Talus nonunion	3	2
Talus osteochondral lesion (OLT)	34	25
Calcaneus nonunion (additional two femoral head allograft were used)	7	5
Navicular nonunion	5	4
Navicular AVN	1	1
Tibiotalar arthrodesis	5	4
Subtalar arthrodesis	12	9
Subtalar + calcaneocuboid arthrodesis 1	1	1
Talonavicular + naviculocuneiform arthrodesis	2	2
Calcaneocubiod arthrodesis	2	2
Tibiotalar + subtalar arthrodesis	4	3
Tarsometatarsal nonunion	1	1
Metatarsophalengeal nonunion (dm. Charchot)	5	4
Talonavicular + subtalar arthrodesis	5	4

TABLE II
Patient demographics, study characteristics and result of treatment

Study	Location	Number of patients and sex	Disease	Mean patient age (range)	Mean body mass index (range)	Comorbidities	Prior surgeries (Y/N)	Mean defect size	Type of original fixation	Mean follow-up (range)	Additional bone graft (Y/N)	Average time to union/ healing (range)	Anastomosis	Complications	Overall % achieving Union (%)
Holm et al.[24]	Navicular	1/F	Traumatic AVN of navicular	48	-	None	Y	3x2 cm	Plate and screws	6 months	N	8 weeks	A dorsalis pedis		100
Cavadas et al.[29]	Distal tibia	20/M 1/F	Distal tibia nonunion	38	-	-	Y	Without bone defect	Plate and screws	-	N	3.1 months	Tibialis posterior	2 Aseptic wound dehiscence 4 Transient numbness in donor site	100
Haddock et al.[23]	Foot and ankle	4/M 1/F	3 talus AVN 1 talus nonunion 1 navicular AVNx	48		None	Y	2x2 cm	1 Cast 1 Ex-fix 1 Plate 2 Screw	20 months	N	23 weeks	Tibialis anterior	None	100
Mattiassich et al.[26]	Talus, calcaneus	2/M	1 talus nonunion 1 calcaneus nonunion	22	-	-	Y	2x2 cm	Cast	3 years	N	12 months	Tibialis posterior 1 flap included adductor magnus tendon for achilles tendon repair	None	100
Caterson et al.[22]	Distal tibia	1/M	Infectious nonunion	52	-	Osteomyelitis	Y	4x2 cm	None	20 months	N	10 weeks	Tibialis posterior	None	100
Hintermann et al.[30]	Talus	9/M 5/F	Talus OLT	35	-	-	13 Y 1 N	1.4x2 cm	11 Screw 3 Cast	4 years	N	12 weeks	-	2 Dysesthesia 3 Additional arthroscopy to the ankle	100
Hsu et al.[20]	Calcaneus	1/M	Calcaneal nonunion Open fracture	26	-	None	Y	4 cm	Screw	36 months	Femoral head and ALT flap	-	Tibialis posterior	None	100
Henn et al.[25]	Distal tibia	1/F 1/M	1 Distal tibia tumor 1 Distal tibia nonunion	49	-	-	Y	2-4 cm	1 Ex-fix 1 Plate	2 years	N	8 weeks	-	None	100
Kazmers et al.[12]	Distal tibia, talus, subtalar	2/M 5/F	1 Osteal tibia 2 Talus AVN 1 Talus nonunion with AVN 3 Talonavicular and subtalar arthrodesis nonunion	38	30	2 diabetes mellitus	Y	-	Plate and screws	14 months	N	15.5 weeks	-	1 Donor site skin debridement	86
Boretto et al.[21]	Tarsometatarsal	1/F	Tarsometatarsal nonunion	65	27	Smoker	Y	2.6 cm		24 months	N	8 weeks	-	Medial condyle osteonecrosis in 23th week	100
Abbate et al.[27]	Metatarsal	1/M	Charchot arthropathy	55	27.3	Diabetic foot peripheral neuropathy osteomyelitis smoker atherosclerosis	Y	4.5x1.5 cm	Ex fix and k-wire	15 months	N	10 months	Dorsalis pedis chimeric flap	None	100
Stranix et al.[17]	Hindfoot (talus talonavicular, talocalcaneal) nonunion and defects	28 patient 30 flaps 14/M 14/F	2 Distal tibia 11 Tibiotalocalcaneal arthrodesis 7 Talonavicular arthrodesis 4 Navicular nonunion 4 Triple arthrodesis 2 Subtalar arthrodesis	47.8 (17-77)	31 (19-45)	13 Smokers 4 Chronic pain 2 Diabetes 1 Peripheral vascular disease 2 Corticosteroid use	19 Y 9 N	2x2 cm	Plate Screw k-wire	15.8 months	-	9 months	23 tibialis anterior 5 tibialis posterior	4 Required soft tissue debulking of the flap 2 Debridement for delayed wound healing at the recipient site 2 Pin removal 1 Tibial screw removal for rod dynamization 1 Excision of heterotopic bone	89

TABLE II
Continued

Study	Location	Number of patients and sex	Disease	Mean patient age (range)	Mean body mass index (range)	Comorbidities	Prior surgeries (Y/N)	Mean defect size	Type of original fixation	Mean follow-up (range)	Additional bone graft (Y/N)	Average time to union/ healing (range)	Anastomosis	Complications	Overall % achieving Union (%)
Saad a et al.[16]	Talus	5 Patients 6 Flaps 4/M, 1/F	Talus avascular necrosis	45	31	None	Y Conventional bone grafting	-	Plate Screw k-wire	20 (8-40) months	N	23.8 (10-52) months	Tibialis anterior	1 Paresthesia	100
Politikou et al.[18]	Foot and ankle	13 Patients 10/M, 3/F	1 Talus OLT 2 Distal tibia 2 Subtalar arthrodesis 3 Tibiotalar arthrodesis 1 Subtalar and calcaneocuboid arthrodesis 2 Talonavicular and naviculocuneiform arthrodesis 1 Calcaneocuboid arthrodesis 1 Ankle and subtalar arthrodesis	45 (29-57)	-	7 Smokers	Y	1.5-3.5 cm	2 None 2 Ex-fix 9 Screw or plate	2.5 years	-	10 months	1 Dorsalis pedis 1 Tibialis posterior 11 Tibialis anterior	8 Numbness donor site	85
Mehta et al.[19]	Calcaneus	1/M	Calcaneal fracture nonunion	25	-	None	Y		Plate and screws	-	Y Femoral head	-		None	100
Windhofer et al.[28]	Talus	19 Patients 11/M, 8/F	Talus OLT	28	27	5 Smokers	10 Y 9 N	1.91 cm^2	2 cast 17 screw	45 months	N	2-6 months	Dorsalis pedis	2 Ankle instability 2 Arthroscopy	100
Madi et al.[33]	Talus	2 Patients	Talus avascular necrosis	-	-	-	Y (core decompression)	-	-	2.75 (2-3.5) years	-	-	-	2 Total talus replacement 1 Below knee amputation	0
Sherman et al.[40]	Tibiotalocalcaneal	3 Patients 3/M	Arthritis, talus avascular necrosis soft tissue defects	54.0	>30	1 Rheumatoid arthritis 3 Osteomyelitis	Y	6.93 (4.5-11.7) cm	3.5 mm Solid screws, MFC was fixated on the femoral head allograft	15 months	Fresh femoral head allograft, demineralized bone matrix, bone marrow aspirate	112 days	Anterior or posterior tibial vessels, end-to-end	0	100
Kaiser et al.[32]	1st MTP	3 Patients 1/F Remaining genders not specified	1 Nonunion, 1 Infection of fusion, 1 Failed 1st MTP arthroplasty	50 (46-57)	29 (23-37)	2 Smokers 2 Post-bacterial infection	Y	Not specified	K-wire 0.65 mm	17 (5-31) months	No	82 (75-88) days	Dorsalis pedis, end-to-end	0	100
Letizia et al.[31]	Talus	1 Patient 1/F	Talus avascular necrosis	26	25	Smoker	Y	2 cm	Press-fit and 2 screws	12 months	No	Unclear, but full weightbearing at 1 year postoperative	Peroneal artery, end-to-end	0	Full weight bearing at 1-year follow-up with no complaints

Y: Yes; N: No; OLT: Osteochondral lesion of talus; ALT flap: Anterolateral thigh flap.

periosteal flap harvesting, and outcomes. All included data was retrieved by a senior surgeon and entered into a computerized database.

RESULTS

Initially, 194 articles were identified, of which 20 met the inclusion criteria and formed the basis of this review.[12,16-33,40] A total of 131 patients (84 males, 43 females; mean age 42±0 years; range, 22 to 77 years) were treated with an MFCF. The sex of four patients could not be retrieved.

The most common clinical indications for the use of the MFCF were osteochondral lesions of the talus (OLT; 37%), distal tibia (pilon) fracture nonunion (22%), failed arthrodesis or nonunions of foot joints (15%), foot bone nonunions (9%), and talus avascular necrosis following index surgery (5%; Table I). Thirty patients were smokers. Five patients had diabetes, five had chronic pain, five had osteomyelitis, two used corticosteroids, and one patient had peripheral vascular disease. Ninety-eight (75%) had undergone previous ankle or foot surgery, with a mean of 3.2±0.1 (range, 1 to 10) previous operations per patient (Table II).

The vessel anastomosis site was reported in 118 cases, and the arteries used were the tibialis anterior artery in 58 patients, tibialis posterior in 31 patients, dorsalis pedis in 25 patients, tibialis posterior or anterior (not specified) in three patients, and peroneal artery in one patient. Union was achieved in 122 (93.1%) patients, whereas nine (6.8%) patients developed nonunion. In three cases where the posterior tibial artery was used for anastomosis, there was complete fusion, resulting in a 100% fusion rate for the posterior tibial artery. However, due to an insufficient number of cases, it cannot be concluded that the posterior tibial artery was the best artery for anastomosis. The mean healing time was 26±0.1 weeks, and the mean follow-up was 24±0.1 months (Table II). Comorbidities of the patients who developed nonunion are shown in Table III.

Following surgery, donor site morbidity was reported in 24 (18%) patients. Overall, 15 patients had postoperative paresthesia at the MFCF harvest site (14 of them had a sensory recovery after a few months),[16,18,29,30] seven patients required additional surgery, three of which required surgical wound revision at the donor site,[12,29] one patient had no sensation on the flap donor site, a 65-year-old female patient developed osteonecrosis of the medial femoral condyle after harvesting of the MFCF that was treated with TKA,[21] and one patient developed heterotopic ossification requiring excision.[17]

During follow-up, additional surgery was performed on 10 patients, two patients were diagnosed with ankle instability after flap surgery due to OLT,[28] five underwent ankle arthroscopy,[29,30] two patients required total talus replacement for persistent pain, and one patient underwent transtibial amputation.[33] After the surgery, five patients underwent joint arthroscopic debridement and synovectomy due to anterior joint impingement, and one of them also underwent joint mobilization under anesthesia.

Radiological evaluation of the donor site was performed in 13 patients using computed tomography and magnetic resonance imaging scans of both knees or preoperative x-rays and angiograms.[18,24,25] No lesions of the medial collateral ligament were observed, but in four cases, the proximal insertion of its superficial part was scarred.[18] There were no fractures of the medial femoral condyle in any of the

TABLE III
Comorbidities in nonunion cases

	Smoker	Diabetes	Osteomyelitis	Previous surgeries	Age	Sex
1	x			3	48	Male
2		x	x	3	51	Female
3				4	45	Female
4	x	x	x	3	34	Male
5				5	61	Male
6	x			2	50	Male
7	x			4	41	Female
8		x		5	55	Female
9	Not specified	Not specified		1	Not specified	Female

patients. Magnetic resonance imaging revealed signs of osteoarthritis in one donor knee,[18] but due to the existence of osteoarthritis in the patient's untreated contralateral knee, it could not be attributed to donor site morbidity.

DISCUSSION

The medial femoral condyle has a well-defined, easily accessible anatomy, gaining its blood supply from the descending genicular artery (89% cases) or the superomedial genicular artery (11% cases), both of which have adequate caliber to allow for successful microsurgical anastomosis, although the descending genicular is preferable due to greater pedicle length.[34] The MFCF, a versatile flap, finds numerous applications in ankle and foot reconstruction. It facilitates the incorporation of diverse tissues to address bone defects, chondral defects, and potential issues related to tendon or soft tissue loss. While primarily employed for small lesions, it can also accommodate larger defects with bone paddles, reaching dimensions of 8×13 cm. With its minimal donor site morbidity and simple surgical dissection, it holds promise as a primary choice for addressing bone nonunions and small bone defects.

When a significant intercalary defect results from resection of diseased bone, successful arthrodesis with limb length preservation requires osseous structural support to bridge the gap in combination with stable compressive fixation. Structural bone grafts require progressive healing through the interfaces between the graft and native bone margins, with larger grafts at higher risk of failure to consolidate.[35] Compromised vascularity of the recipient site and comorbidities such as diabetes, osteomyelitis, or smoking further increase the risk of graft failure.[36,37] Cavadas and Landín[29] achieved successful vascularization by applying MFCF in cases of nonunion in the distal tibia region, resulting in successful union in all cases. It is a favorable choice not only for cases with tissue defects but also for those with decreased biological vascularity in nonunion cases. Vascularized bone grafts have the advantage of preserving osteocyte viability within the structural bone graft transferred.

Nonunion following arthrodesis surgery is associated with poor function, disability, and the potential need for revision surgery. A number of factors have been reported to be associated with nonunion, including patient factors, local factors at the site of surgery, and surgical factors.[4]

While the use of allografts leads to high union rates, the absence of vasculature can lead to infection or resorption. These recalcitrant cases, particularly poorly vascularized atrophic nonunions and arthrodesis nonunions of the ankle joint, are significantly more challenging to resolve, with further nonvascularized bone grafts resulting in poor outcomes.[3] In this case, a vascularized transfer may be required to achieve successful union. Vascularized periosteal flaps have been identified as a potential solution, as they can be wrapped around the fracture/nonunion site rather than being inserted into the defect as vascularized structural support. Interestingly, in the foot and ankle area, the size of the defect generally does not exceed 6 cm, and these defects require a small flap with a good blood flow, such as the MFCF. An MFCF should not be considered for defects larger than 6 cm. Instead, a free vascularized fibula would be a better option in such situations.

Masquelet et al.[38] described that the medial femoral condyle periosteum is a suitable donor site in humans. The use of the MFCF in scaphoid nonunion was previously described,[8] and Zhou et al.[14] published a systematic review about the outcomes of treatment MFCF in scaphoid nonunions. It could be taken along with the periosteum, had low donor site morbidity, and high success rates. The inner cambium layer of the periosteum facilitates bone growth. The cambium layer is the site of osteogenic activity of the periosteum that produces osteoprogenitor cells for bone repair and growth.[39] The outer layer of the periosteum plays less of an osteogenic role in adults. The inner cambium layer should be protected from damage by harvesting a thin cortical portion of the bone, thereby raising a corticoperiosteal rather than a periosteal-only flap. Sherman et al.[40] have stated that it can be used in tibiocalcaneal arthrodesis in this region and has high fusion rates.

Weir et al.[9] published a systematic review on MFCFs in recalcitrant long bone nonunions. In the study, free vascularized periosteal flaps demonstrated a 99% success rate in achieving union in difficult long bone nonunions compared to 80% for standard orthopedic methods. Despite their promise and the advantages of low donor site morbidity and high success rates, more robust studies with better design and larger patient numbers are needed to confirm these findings. Both of the systematic studies showed that MFCF has a high success union rate in these problematic regions;[9,14] however, its role in the ankle and foot region has yet to be fully elucidated.

Arthrodesis in the foot and ankle region is an option where anatomical reconstruction cannot be achieved. A failed arthrodesis that leads to nonunion might necessitate free vascular flaps as a salvage option. Most of the patients who underwent MFCF had a history of previous surgery. In this study, 98 of the 131 patients whose previous surgery was recorded had undergone previous surgery, with a mean number of 3.2±0.1. Hence, it appears that MFCF is used as a salvage procedure in cases with repetitive previous surgeries that were associated with failure.

All OLT patients (n=33) showed radiological and functional improvement. However, two patients developed ankle instability due to the surgical exposure. All five patients with restricted range of motion regained their movement after ankle arthroscopy. Functional and radiological improvement was also reported in OLT patients who underwent MFCF. The MFCF was applied together with cartilage flap in 33 OLT patients and with skin island as chimeric in five patients. It was taken together with the adductor tendon, which was used for Achilles tendon repair in one patient. It was used together with anterolateral thigh flap in two cases, and a femoral head allograft was used for calcaneus reconstruction in two cases. Good results were obtained in all different combinations.[18,28,30]

The MFCF had an early postoperative complication rate of 29.7%. In nine patients, the numbness recovered early; therefore, we can state that the actual complication rate is 22%. Twenty-one patients needed revision surgery, with 10 of them requiring it at the treatment site. When surgeries due to wound closure problems and the number of flap thinning procedures are excluded from the total revision surgery count, the actual complication rate is observed to be 7%. The MFCF was found to have a 93% union rate, supporting the view that it can have excellent results when used in the foot and ankle region. A number of factors have been associated with nonunion, including patient factors, local factors at the site of surgery, and surgical factors.[3] All the patients in this study had one or multiple comorbidities, except for two patients (Table III).

Overall, based on the evidence obtained from this study, some observations can be made about the use of MFCF in foot and ankle reconstruction. The MFCF is a flap with good blood supply and vascularity supporting osseointegration. The cambium layer should be preserved. Thus, when harvesting the flap, one should not only include the periosteum but also a part of its underlying cortical layer. The MFCF's versatile anatomy makes it suitable for specification in a defect-specific way. The periosteum, cortical bone, cartilage, tendon, and skin can be harvested together or separately from this area, an advantageous feature for the reconstruction of complicated cases. Examples of uses in cartilage defects include scaphoid lesions, Kienböck's disease, and OLT.[18,28,30] The flap is easily accessible due to its simple anatomy, while its harvesting phase is much faster and safer than other free flaps. Moreover, if the donor site does not exceed 6 cm, complication rates remain low, and the biomechanics of the knee joint are not affected after harvesting. Although it has the lowest morbidity rate among free flaps, the most common complication is numbness at the donor site. Numbness was observed in 15 of the 131 patients in this study. Fourteen of the patients recovered spontaneously; however, one of them had a persistent sensory defect at the skin of the donor area.

The MFCF is the first choice for small defects, and this is a common point emphasized in all the studies. It is very effective in places with no or small defects, insufficient blood supply, or soft tissue compromise, a situation often encountered in the foot and ankle region. It should be considered the first choice of treatment in cases of nonunions after recurrent surgeries. The MFCF is not a full cortical graft like the vascularized fibula; thus, the corticoperiosteal diameter can be adjusted and shaped by wrapping around the non-union zone. It has a pedicle with a length of 7 to 9 cm, a suitable length for anastomosis. We observed that the majority of the anastomoses were made to the tibialis anterior, but that is mainly dictated by the localization of the lesion. If the lesion is located in the foot region, then the dorsalis pedis artery is preferred. In this study, the location of the anastomosis was specified in 118 of the cases. Lastly, flap fixation was done as dictated by the localization of the lesion. One screw or K-wire was preferred in the talus in cases of OLT, while plates and screws were preferred in arthrodeses and distal tibia. In some cases, fixation material was not used, and they were managed with an external fixator or plaster.

In conclusion, the MFCF can be considered an optimal graft for tissue reconstruction in the foot and ankle region. It has excellent union rates in this problematic area, is simple to harvest, and is mostly appropriate for smaller bone defects (<6 cm). The MFCF provides good blood flow and favors osseointegration with low complication rates.

Ethics Committee Approval: This narrative review is a literature review, and ethical committee approval is not required. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: Since this study is a review of the existing literature, patient consent is not required.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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