Panagiotis Dragonas, Theodoros Katsaros, Julie Schiavo, Pablo Galindo-Moreno, Gustavo Avila-Ortiz

Osteogenic capacity of the sinus membrane following maxillary sinus augmentation procedures: A systematic review

KEY WORDS

osteogenic, sinus elevation/graft, sinus membrane, systematic review

ABSTRACT

Purpose: The evidence pertaining to the contribution of the sinus membrane to new bone formation following maxillary sinus augmentation procedures is equivocal. The purpose of this study was to analyse the evidence currently available on the osteogenic capacity of the sinus membrane following maxillary sinus augmentation procedures, and the effect of local delivery of recombinant human bone morphogenic proteins (rhBMPs) on the bone-forming potential of the sinus membrane.

Materials and methods: An electronic search was conducted using six different databases to identify controlled trials, prospective and retrospective cohort studies, case series and case reports, as well as preclinical (animal) studies reporting on new bone formation in close proximity with the sinus membrane after maxillary sinus augmentation procedures, assessed through histological and/or histomorphometrical evaluation, on the basis of pre-established eligibility criteria.

Results: No clinical studies were identified. Twenty-six preclinical studies were included in the review. Nine of them supported the osteogenic potential of the sinus membrane, while eight reported no evidence of osteogenicity from the sinus membrane. The nine remaining studies reported on the local effect of rhBMPs. The majority of these nine studies reported enhanced new bone formation in the sinus membrane region.

Conclusions: The sinus membrane contains pluripotent mesenchymal cells with the capacity to differentiate and participate in the process of new bone formation. However, the findings from the studies selected in this systematic review do not consistently support that the sinus membrane significantly contributes to new bone formation following maxillary sinus augmentation procedures.

Conflict-of-interest statement: *The authors declare no conflicts of interest.*

Introduction

Management of vertical bone deficiencies in the posterior maxilla often represents a significant challenge in the context of tooth replacement via dental implant therapy due to the proximity of the maxillary sinus, also known as the antrum of Highmore. Maxillary sinus augmentation (MSA) procedures are one of the most indicated therapeutic alternatives for implant site development in this anatomical location. Elevation of the sinus membrane (SM) via a lateral or crestal approach, with or without the addition of bone grafting materials, has been associated with new bone

formation (NBF) in the antral cavity[1,2]. Interestingly, NBF has been demonstrated histologically in close proximity to the bony floor of the maxillary sinus and the elevated SM during the early stages of healing in preclinical models[3,4]. This raises the question of the extent to which the SM contributes to the osteogenic process in MSA procedures.

From a histological point of view, the SM has been classically described as a structure composed of three main layers: a ciliated pseudostratified epithelium that faces the antrum, a highly vascularised lamina propria and, interfacing with the bony boundaries of the maxillary sinus, a layer that has been described as a periosteum-like structure[5]. Since periosteum has been shown to host osteoprogenitor mesenchymal cells that contribute to NBF after elevation of the periosteal lining[6], it has been hypothesised that bone regeneration following MSA could be partially related to an osteogenic response associated with the SM[5]. Nonetheless, this concept has been challenged by Lin et al[7], who reported that the periosteal portion of the membrane histologically differs from the periosteum that covers the maxillary and mandibular alveolar ridges (also Sharawy E and Misch CE, unpublished data, 2007).

A number of in vitro studies have assessed the osteogenic potential of SM cells[5,8-15]. Kim et al[9] and Graziano et al[11] reported that human SM mesenchymal stem cells can be differentiated into osteoblasts at as early as 30 days when cultured in osteogenic medium. Srouji et al[5,10] also assessed whether the SM contains an osteogenic progenitor cell population. Cells were cultured first in non-osteogenic medium, which resulted in expression of multiple progenitor cell markers. After an additional 4 weeks in osteogenic medium, the cells showed typical osteogenic cluster formation, expressing alkaline phosphatase (ALP), osteocalcin and mineral deposition. Finally, Yun et al[12] cultured human SM stem cells in non-osteogenic media. They reported no osteocalcin expression and no calcified nodule formation. Once these cells were treated with simvastatin, which has been reported to enhance NBF, they were characterised by expression of bone morphogenetic protein 2 (BMP-2), osteocalcin, ALP activity and mineralisation. The authors suggested that SM stem cells may not have osteogenic potential by themselves, but need the presence of an osteogenic environment to induce NBF.

In a preclinical study, Palma et al[3] reported NBF in direct contact with the SM following graftless MSA with simultaneous implant placement in a preclinical primate model, which, according to the authors, was indicative of the osteoinductive potential of the SM. However, following another study in primates that was primarily aimed at assessing early healing events after graftless MSA (i.e., blood coagulum alone), Scala et al[16] reported that new woven bone sprouted only from the existing bony boundaries. Similarly, Jungner et al[17] reported that bone formation after SM elevation is initiated from the sinus floor and sprouts into the space created after SM elevation, without any evidence of the SM having osteoinductive potential.

The potential effects of locally delivered recombinant human bone morphogenic proteins (rhBMPs) on the osteogenic response of the SM have also been addressed in the literature. In a preclinical study, Cha et al[18] observed enhanced bone formation in the peripheral portion of the antral cavity, in direct contact with the SM, at 20 weeks after MSA using rhBMP-2 in a bovine particulate xenograft and a collagen carrier compared to control sites that underwent the same intervention, but in the absence of rhBMP-2.

Therefore, the aim of the present systematic review was to critically assess the evidence pertaining to the osteogenic role of the SM in MSA procedures, with additional emphasis placed on evaluating the effect of locally delivered rhBMPs on the bone-forming potential of the SM.

Materials and methods

This systematic review was structured and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines[19]. The following focused question was formulated: 'Does the sinus membrane (SM) contribute to new bone formation (NBF) following maxillary sinus augmentation (MSA) procedures?'

Outcome of interest

The outcome of interest was the presence of NBF in close proximity to the SM after MSA procedures, assessed through histological and/or histomorphometrical evaluation.

Eligibility criteria

Articles reporting the results of human randomised and non-randomised controlled trials, prospective and retrospective cohort studies, case series and case reports, as well as preclinical/animal studies involving transcrestal or lateral MSA procedures with or without the concomitant use of bone grafting material, were eligible for inclusion. Participants in clinical studies included human adult patients (> 18 years of age) who underwent MSA involving delayed implant placement and histological/histomorphometrical analysis of augmented bone after a variable healing period (3 to 12 months). Only articles that reported the aforementioned outcome variables were eligible. Studies were excluded if NBF in direct contact with the SM after MSA was not specifically assessed.

Search strategy

A literature search was conducted using six databases: Ovid MEDLINE, Scopus, Embase, Central (Cochrane Library), Web of Science and ProQuest (Dissertations and Theses and Nursing and Allied Health Database) up to 11 December 2019. No other date limits were applied. Only studies published in English were included. Medical subject headings (MeSH) and key terms included (sinus floor augmentation OR maxillary sinus augmentation OR sinus lift OR sinus elevation) AND (nose mucosa OR nasal mucosa OR sinus mucosa OR Schneiderian membrane OR sinus membrane) AND (bone development OR ossification OR bone regeneration OR osteoprogenitor cell OR osteogenic potential OR osteogenic differentiation OR osteoinductive OR osseous regeneration OR bone ossification OR bone formation OR osteoclastogenesis). Given the narrow scope of the topic, the grey literature was not searched. The MEDLINE search was adapted for use in searching the other databases. The search was supplemented by citation screening and scanning of all reference lists of selected papers.

Screening and selection of studies

The titles and abstracts obtained were independently screened by two of the authors (PD and TK) based on the eligibility criteria described above. If insufficient information was provided to make a decision to exclude an article, the article was included for full-text review. Full-text versions of all potentially eligible articles based on initial screening were obtained and independently examined by both reviewers for final selection. Any disagreements were resolved by open discussion. If a disagreement could not be resolved, an arbiter (GA) was consulted.

Data extraction

All selected articles were subdivided into three separate tables: studies supporting contribution of the SM to NBF, studies not supporting contribution of the SM to NBF and studies on the local effect of BMPs on the osteogenic capacity of the SM. Two of the authors (PD and TK) independently extracted the relevant data using a pre-designed data extraction table. The data extracted for the animal studies included author, study purpose, number and type of animals, procedure, timing of sacrifice and biopsy specimen, histological outcomes and conclusions. The data extracted for the clinical studies included author and year of publication, study design, population characteristics, parameters recorded, summary of the methodology, technical details of the surgical intervention, comparison/control and treatment outcomes. A decision was made to report separately on animal studies that used BMPs in their protocol, as their presence might alter the healing pattern of NBF and enhance the osteogenic potential of the SM when compared to non–BMP-treated sites[20-22].

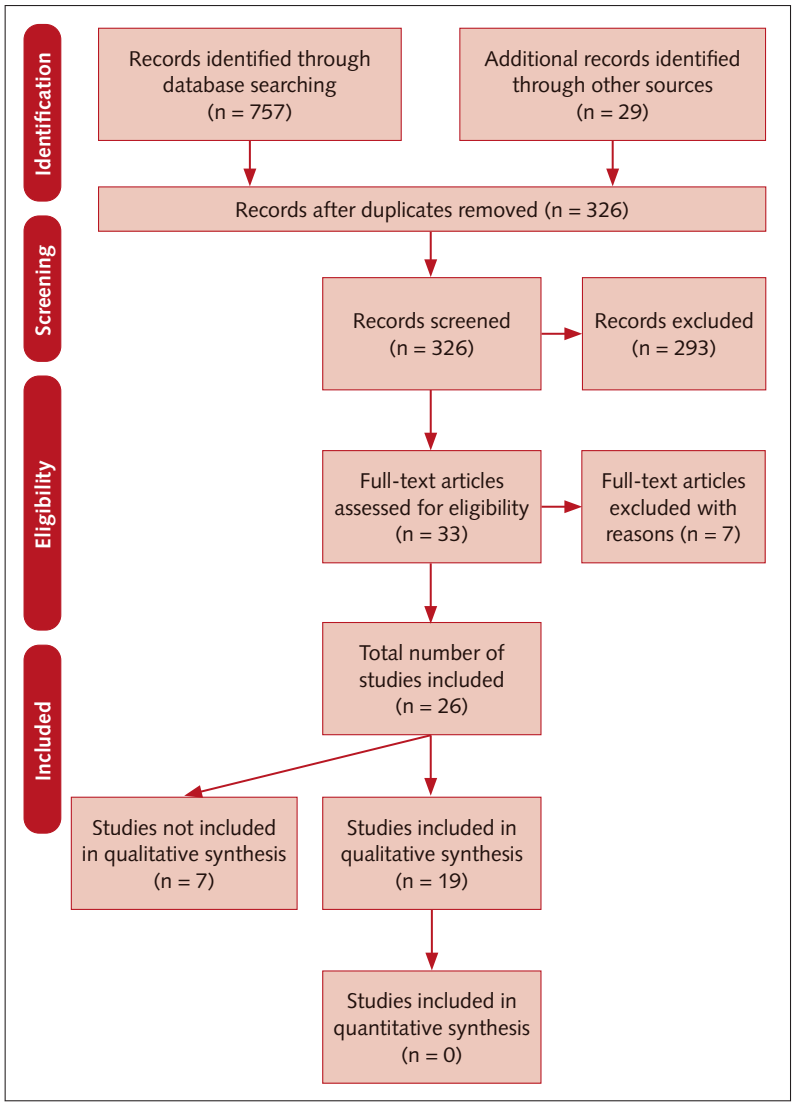

Fig 1 Flow diagram of article selection process.

Risk of bias and quality assessment of included studies

The methodological quality of interventional trials was evaluated according to the Cochrane Collaboration's tool for assessing risk of bias[23], adapted by Chambrone et al[24,25] to permit qualification of non-randomised trials. The randomisation and allocation methods, blinding of patients and examiners, completeness of follow-up, selective reporting and other sources of bias were classified as adequate (+), inadequate (-), unclear (?) or not applicable (NA). Based on this tool, the risk of bias was classified as low if all criteria were met; unclear if one or more of the criteria were unclear; and high if one or more of the criteria were not met.

For interventional animal studies, the methodological quality of the trials was evaluated using the SYRCLE (SYstematic Review Center for Laboratory animal Experimentation) risk of bias tool for animal studies[26]. This tool is based on the Cochrane risk of bias tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies. The following domains were addressed: sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers and researchers, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting and other sources of bias. Each was classified as "Yes/(+)", indicating a low risk of bias, "No/(-)", indicating a high risk of bias, "Unclear/(?)", indicating an unclear risk of bias or "N/A", not being applicable to the study. Based on this tool, the risk of bias was classified as low if all domains were at low risk of bias, high if one or more domains were at high risk of bias, and unclear if one or more domains were at unclear risk of bias.

Results

Study selection

The article selection process is depicted in Fig 1. A total of 326 potentially eligible articles were identified once duplicates were removed. Following application of the eligibility criteria, 293 articles were excluded. After a review of the remaining full-text articles, 7 articles were excluded for multiple reasons (Table 1). A total of 26 animal studies were included, 19 of which were controlled. As the SYRCLE risk of bias tool is designed for controlled animal studies, qualitative analysis was performed on the 19 animal studies that were identified as controlled. It is notable that no clinical studies meeting the inclusion criteria were identified.

Table 1 Excluded studies and reasons for exclusion

Study	Reason for exclusion
Sul et al[49]	Authors did not specifically comment on lack of bone between implant apices and SM and how this associates with bone-forming abilities/lack thereof of SM
Schweikert et al[50]	Use of titanium device for space maintenance purposes was ineffective and perforated SM, thus study outcomes were questionable
De Santis et al[51]	Authors report presence of new bone in the elevated sinus area but do not comment on the origin of the bone (SM or parent bone walls)
Lim et al[52]	Histological analysis of different SM regions referred to number of new blood vessels and not NBF
Kim et al[53]	Authors do not elaborate on the histological findings in the submucosal area, despite presenting histological images of the area
Hwang et al[54]	Authors do not elaborate on the histological findings in proximity to SM, despite presenting histological images of the area
Masuda et al[55]	Authors do not elaborate on the histological/morphometrical findings in the submucosal area, despite identifying it as a region selected for analysis in their methodology

Risk of bias and quality assessment of included studies

The quality assessment of all included animal studies is presented in Table 2. Out of the 19 studies, 9 were identified as having a high risk of bias and 10 as having an unclear risk of bias, based on the previously described criteria.

Animal studies supporting osteogenic potential of the SM

Nine studies were identified that reported evidence of participation of the SM in NBF (Table 3).

In a study on primates where sinuses were filled with either autogenous bone or coagulum only, Palma et al[3] reported the presence of NBF in contact with the SM in non-grafted sites 6 months post-augmentation. They claimed that such findings confirm the osteoinductive potential of the SM and may be explained by the presence of SM cells with osteogenic potential.

In another study on primates, Cricchio et al[27] reported the presence of new bone in intimate

Table 2 Qualitative analysis of the included controlled animal studies

	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding of caregivers and researchers	Random outcome assessment	Blinding of outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Sohn et al[32]	?	+	?	+	N/A	N/A	?	+	+	+
Kim et al[20]	-	+	-	+	N/A	N/A	?	+	+	+
Lim et al[42]	+	+	+	+	N/A	N/A	+	+	+	+
Yoon et al[43]	-	+	+	+	N/A	N/A	?	+	+	+
Hong et al[41]	?	+	?	+	N/A	N/A	?	+	+	+
Wada et al[44]	-	+	-	+	N/A	N/A	?	+	+	+
Yon et al[21]	+	+	+	+	N/A	N/A	+	+	+	+
Cha et al[18]	-	+	-	+	N/A	N/A	+	+	+	+
Kim et al[40]	?	+	?	+	N/A	N/A	?	+	+	+
Choi et al[22]	?	+	?	+	N/A	N/A	?	+	+	+
Favero et al[39]	?	+	?	+	N/A	N/A	?	+	+	+
Jungner et al[17]	-	+	-	+	N/A	N/A	?	+	+	+
Scala et al[38]	?	+	?	+	N/A	N/A	?	+	+	+
Fuerst et al[33]	-	+	-	+	N/A	N/A	?	+	+	+
Sohn et al[4]	?	+	?	+	N/A	N/A	?	+	+	+
Sohn et al[31]	-	+	-	+	N/A	N/A	?	+	+	+
Rong et al[30]	-	+	-	+	N/A	N/A	+	+	+	+
Moon et al[28]	?	+	?	+	N/A	N/A	?	+	+	+
Palma et al[3]	-	+	-	+	N/A	N/A	?	+	+	+

Table 3 Studies supporting osteogenic potential of the SM

Study	Purpose	Number and type of animals	Procedure	Timing of sacrifice and biopsy specimen	
Palma et al[3]	To compare histological outcomes of MSA with DI and with/without autogenous bone grafts	4 male tufted capuchin primates	Bilateral MSA-L (window repositioned at the end) with simultaneous DI. One sinus filled with autogenous bone (T), the other with coagulum (C)	6 mo	
Cricchio et al[27]	To assess whether a resorbable dome can be used to elevate the SM and form bone prior to DI	8 male tufted capuchin primates	MSA-L (window repositioned at the end). All animals received either an H-shaped or star-shaped space-making device (polylactide 70:30) to keep SM elevated	6 mo	
Moon et al[28]	To compare the effect on NBF of replaceable bony windows and resorbable collagen membranes over bone grafts	16 male New Zealand white rabbits	Bilateral MSA-L. C: β-TCP was grafted under SM and collagen membranes covered lateral window. T: β-TCP was grafted under the SM and bone windows were replaced	1, 2, 4 and 8 wk	
Lim et al[29]	To compare volume stability and NBF capacity between BCP with a higher ratio of β-TCP (30:70) and a lower ratio of β-TCP (70:30)	8 adult New Zealand white rabbits	Bilateral MSA-L (one with 70:30 and the other with 30:70)	2 and 8 wk	
Rong et al[30]	To find out whether the SM is involved in NBF	12 female beagle dogs	Bilateral MSA-L (3 groups). Control (C): SM elevated and xenograft placed. Mucosal shielding group (MS): Ultrathin titanium membrane implanted against SM and then xenograft placed. Bone wall shielding group (BWS): Ultrathin titanium membrane placed against surrounding bone after SM lifting to block the medial, anterior, posterior and inferior walls, then folded to block the buccal wall after xenograft placed. Window replaced before closure. At 2 mo, the same procedure was done on the other side	1 and 3 mo	
Sohn et al[31]	To assess the remodelling process of NBF after SM elevation, either alone or with bone grafting	20 adult male New Zealand white rabbits	Bilateral MSA-L. Ungrafted group: Titanium miniscrew inserted to support SM and bone window repositioned. Grafted group: Xenograft placed under the SM and collagen membranes at bone window. PCNA, type I collagen and osteocalcin were used to demonstrate the area and intensity of NBF in the sinus	1, 2, 4, 6 and 8 wk	

	Histological outcomes	Conclusions
	A strip of bone could be frequently seen lining SM near the uppermost part of the implant. C: NBF at the periphery in contact with SM and extending downwards to the centre of the augmented area and in contact with the DI. T: Bone tissue was seldom seen lining SM at the implant apex and resembled a sequestered island encapsulated by fibrous tissue trapped between the DI and SM during graft insertion	Presence of NBF in contact with SM in non-grafted sites confirms the osteoinductive potential of the membrane
	SM was found in intimate contact with NB tissue. NBF was a common finding in all sinuses irrespective of the type of device. As a general rule, trabecular bone originating from the sinus periphery was projected into the centre in the vast majority of cases	Results from the present study suggest osteoinductive properties of SM
	T and C (same): 1 wk: NBF observed on the surface of the elevated SM and around lateral wall of the maxillary sinuses; 2 wk: More active NBF observed along the β-TCP particles on the surface of the elevated SM and around lateral wall of the maxillary sinuses; 4 wk: NBF highly increased along the β-TCP particles on the surface of the elevated SM and around lateral wall of the maxillary sinuses. T: Many TRAP-stained osteoclasts on the replaced bony windows, elevated SM and lateral wall of the maxillary sinuses revealed early in the healing period, indicating active NBF. C: TRAP-stained osteoclasts not seen along the collagen membranes, indicating no bone regeneration early in the healing period. Some were seen along the elevated SM and around lateral wall	Elevated SM in both groups showed osteoinductive efficacy
	2 wk: NB had formed adjacent to the SM and in contact with the graft particles in both groups; 8 wk: Substantial NBF observed in both central and SM areas	N/A
	1 mo: C: Collagen synthesis around particles adjacent to the bony wall, with a large number of osteoprogenitor cells and osteoblasts. Particles close to SM were enveloped in fibrous connective tissue with few areas of collagen synthesis around particles where osteoprogenitor cells were found. MS: Area close to the bony wall showed a similar pattern to that in C. Particles adjacent to SM were enveloped by fibrous connective tissue, with no notable collagen synthesis or osteoprogenitor cells. BWS: Particles adjacent to the bony wall were enveloped by fibrous connective tissue with no sign of collagen synthesis. Area close to SM showed a similar pattern to that in C. 3 mo: C: Large amounts of NB around particles adjacent to the bony wall. Signs of cartilage and bone around particles adjacent to SM. MS: Particles adjacent to bony walls were in a pattern similar to those in C. Most particles adjacent to SM were enveloped in loose connective tissue together with a few areas of chondroid tissue. The overall amount of NBF differed only slightly from that in C. BWS: Particles adjacent to the bony wall were enveloped by much fibrous connective tissue with osteoprogenitor cells locally. Particles adjacent to SM were surrounded by newly formed bone tissue with signs of cartilaginous lacunae, osteocytes and bony lacunae	SM has osteogenic capability and is involved in NBF after the floor of the sinus has been raised. However, its osteogenic potential is weaker than that of the surrounding bony walls of the maxillary sinus
	Ungrafted sites: 1 wk: NBF along the repositioned bony window and the elevated SM; 2 wk: NBF expanded from the elevated SM to the centre; 4 wk: Sinus cavity filled with NB and fibrous tissue with abundant blood vessels; 6 wk: NB thicker than 4-wk samples. Grafted sites: 1 wk: A bit of NB along the elevated SM; 2 wk: NB was found mainly along SM; 4 wk: NB bound to xenograft particles; 6 wk: More mature and abundant NB was found; 8 wk: Xenograft surrounded by dense and mature NB. Immunochemical findings: PCNA expression: Ungrafted: 1 and 2 wk: Strong expression of PCNA in osteoblasts along the elevated SM; 4, 6 and 8 wk: Positive cells for PCNA but intensity was weaker than in the 2-wk group. Grafted: 1 wk: PCNA+ cells hardly observed, with few at the NB formed under SM; 2 wk: strong + staining for PCNA in particles and NB; 4, 6 and 8 wk: Much weaker expression of PCNA+ cells. Type I collagen: Ungrafted: 1, 2 and 4 wk: Strong expression of type I collagen on surfaces of NB and bone-forming osteoblasts under SM. Grafted: 1 wk: Slightly positive expression of type I collagen under SM; 2 wk: Strong expression of type I on osteoblasts and NB–forming cells around particles. Expression of osteocalcin: Ungrafted: 1 wk: Slight expression around osteoblasts on the surface of the elevated SM; 2 wk: Strong expression around NB on SM; 4 wk: Strong expression on surface of NB; 6 and 8 wk: similar to 4 wk. Grafted sites: No report on expression below SM	NBF started from the inner surface of the elevated SM and the floor of the repositioned bone window and progressed to the central portion of the sinus as healing time increased. Repositioned bone window and SM act as starting points to induce NBF in the early stages of sinus augmentation

Table 3 (cont.) Studies supporting osteogenic potential of the SM

Study	Purpose	Number and type of animals	Procedure	Timing of sacrifice and biopsy specimen	
Sohn et al[4]	To assess the process of NBF in MSA with and without bone grafting	20 adult male New Zealand white rabbits	Bilateral MSA-L. T: Bone window removed and replaced after SM elevation without bone grafting. A titanium mini screw was inserted to support the elevated SM. C: Xenograft under the elevated SM membrane and CM on lateral window	1, 2, 4, 6 and 8 wk	
Fuerst et al[33]	To determine whether autogenous cells added to bovine bone mineral enhance NBF after MSA	5 adult minipigs	Bilateral MSA-L. ABCs from bone biopsies were isolated, cultured, stored and then mixed with BBM (ABC + BBM) (T). BBM + saline (C). One DI per sinus was placed simultaneously. 3 zones in sinus area analysed separately (BW, M, SM)	12 wk	
Sohn et al[32]	To compare bone regeneration after MSA using graft materials and demineralised tooth dentine	18 adult male New Zealand white rabbits	Bilateral MSA-L. Group 1 (C): blood clots; Group 2: anorganic bovine graft; Group 3: β-tricalcium phosphate (β-TCP); Group 4: demineralised tooth dentine	2, 4 and 8 wk	

ABC, autogenous bone cell; BBM, bovine bone mineral; β-TCP, beta-tricalcium phosphate; BW, near the facial bony wall; BWS, bone wall shielding group; C, control; DI, dental implant; M, middle zone; MS, mucosal shielding group; MSA-L, maxillary sinus augmentation with lateral window; NB, new bone; NBF, new bone formation; PCNA, proliferating cell nuclear antigen; T, test; TRAP, tartrate-resistant acid phosphatase; SM, sinus membrane; SSD, statistically significantly different.

contact with the SM 6 months post-augmentation, using only a dome to support the elevated SM. As a general finding, they also noted new trabecular bone originating from the sinus periphery and projecting to the centre of the augmentation area. They claimed that such findings suggest that the SM has osteoinductive properties.

In a study on rabbits, Moon et al[28] observed NBF immediately apical to the SM at 1 week after lateral MSA with β-tricalcium phosphate (β-TCP), which significantly increased at weeks 2 and 4. They noted the presence of tartrate-resistant acid phosphatase (TRAP)–stained osteoclasts early in the healing period, indicating active bone turnover in direct contact with the SM. Lim et al[29], using the same animal model, observed NBF adjacent to the SM as early as 2 weeks following lateral MSA using different ratios of β-TCP.

In a study on beagle dogs, Rong et al[30] aimed to assess whether the SM is involved in NBF post–sinus augmentation using a xenograft. More specifically, they evaluated the extent of the contribution of the SM and host bone to NBF by placing titanium meshes either against the SM (MS, mucosal shielding group) or the surrounding bone (BWS, bone wall shielding group) in order to exclude any NBF associated with them, and compared histological outcomes to a control (C) group where only a xenograft was used below the SM. At 3 months, in the C group, large amounts of new bone were found around particles adjacent to the host bone, with signs of cartilage and bone adjacent to the SM. In the MS group, most particles adjacent to the SM were enveloped in loose connective tissue, whereas no difference was noted proximal to the host bone when compared to the C group. In the BWS group, particles adjacent to the bony walls were enveloped by fibrous connective tissue, whereas particles adjacent to the SM were surrounded by newly formed bone. For the MS group, the authors noted that the overall amount of new bone differed only slightly from that for the C group, and concluded that the SM is involved in NBF after sinus floor elevation. However, its osteogenic potential is weaker compared to the surrounding bony walls.

Sohn et al[4,31], in two similarly designed studies on rabbits, noted NBF as early as 1 week post–sinus augmentation, originating from the elevated SM and progressing to the central portion of the

	Histological outcomes	Conclusions
	T: 1 and 2 wk: NB formation under SM and floor of the replaced bone window. C: 1 wk: NBF under SM. More active NBF along elevated SM than along xenograft particles; 4 wk: NBF increased too, and some NB was bound to the xenograft particles. Greater NBF observed on SM than on xenograft particles	Bone remodelling process started from the elevated SM, the floor of the sinus cavity and the replaced bony window. Greater NBF on SM and floor of the repositioned bony window than at the central area of sinus, indicating osteoinductive properties of SM
	NBF more pronounced near BW (local host bone) of the sinus than in the zone near SM. Overall, NB: 29.86 ± 6.45% (T) vs. 22.51 ± 7.28% (C). In T, 37.23 ± 8.24% NB was found near BW vs. 31.63 ± 7.74% in the M and 20.7 ± 4.5% near SM. In C, corresponding %s were 28.42 ± 12.54 (BW), 22.5 ± 7.91 (M) and 15.43 ± 3.62 (SM). SSD for M and SM areas	ABCs possess osteogenic potential and therefore increase NBF in regions with a low number of bone-forming cells including middle and SM regions
	NBF under the surface of the elevated SM in Groups 1, 2 and 3 as early as 2 wk, which increased at 4 and 8 wk	N/A

augmented region as healing time increased. Immunochemical findings revealed expression of proliferating cell nuclear antigen (PCNA), type I collagen and osteocalcin in the osteoblasts disposed over the elevated SM, demonstrating areas of NBF activity[31]. They noted that the SM acts as a starting point for NBF in the early healing stages after MSA, thus exhibiting osteoinductive properties. Using the same animal model, Sohn and Moon[32] also noted NBF in the submucosal area as early as 2 weeks post-augmentation, irrespective of the bone grafting material used, or whether a graft was used or not.

Finally, Fuerst et al[33] assessed the effects of adding autogenous bone cells (ABC group) to a xenograft in MSA, using a minipig model. They observed that there was an increase in NBF in regions with an inherently low number of bone-forming cells, i.e. the centre of the sinus augmented region and the SM. More specifically, 12 weeks post–sinus elevation, the proportion of NBF adjacent to the SM was 20.7 ± 4.5% for the ABC group, which was significantly higher than in the xenograft-only group (15.43 ± 3.62%).

Animal studies not supporting osteogenic potential of the SM

Eight studies were identified as reporting no evidence of osteogenic properties for the SM (Table 4).

In two studies on primates where lateral MSA with simultaneous implant placement and no grafting was performed, Scala et al[16,34] reported that the SM was unable to induce NBF during the early stages of healing. They noted that intrasinusal NBF started from the bony walls, with no evidence of NBF under the SM or above the implant apex.

Similar outcomes were reported by Qian et al[35]. In a study on dogs assessing bone formation after osteotome MSA and simultaneous implant placement with no grafting, they found new bone sprouting from the parent bone walls. In contrast, NBF could hardly be observed on the implant apex, immediately below the SM.

Scala et al[36] and Jungner et al[17] assessed early healing events after MSA in minipigs and sheep respectively, with autogenous bone used as graft material. They reported similar healing patterns to other studies in which only coagulum was allowed to fill in the space. Specifically, NBF started at the

Table 4 Studies not supporting osteogenic potential of the SM

Study	Purpose	Number and type of animals	Procedure	Timing of sacrifice and biopsy specimen	
Scala et al[16]	To assess early healing of MSA with DI and no filling material	8 male tufted capuchin primates	MSA-L (window reflected inside sinus cavity) with simultaneous DI (protruding about 5.7 mm into the void). Coagulum filled the sinus cavity	4, 10, 20, 30 d	
Scala et al[34]	To assess early healing of MSA with DI and no filling material	8 male tufted capuchin primates	MSA-L with simultaneous DI. Coagulum filled the sinus cavity. Window closed with demineralised bovine cortical bone	4, 10, 20, 30 d	
Scala et al[36]	To assess healing of MSA after elevation of SM with use of autogenous bone and no CM occluding the osteotomy access	10 minipigs	MSA-L. Autogenous bone in sinus cavity after SM elevation. No CM on lateral osteotomy. Bone window reflected into the sinus cavity	15, 30, 90, 180 d	
Scala et al[38]	To assess the influence of CM placed subjacent to SM on the healing outcome of MSA	8 Pelibuey sheep	Bilateral MSA-L. Window outlined and removed. CM was applied below the SM only at test (T) with the opposite side left without CM (C). Space created below SM filled with xenograft. CM was positioned to cover the access osteotomy on T and C. 3 regions analysed (base, middle, SM)	4 mo	
Jungner et al[17]	To assess early bone formation in MSA 10 to 45 d after elevation	9 male tufted capuchin primates	8 (T) – 1 (C) Bilateral MSA-L (window repositioned at the end). 2 time sequences: 10 and 45 d. 4 animals/8 sinuses at each time sequence (2 perforated sinuses, 4 sinuses with SM elevation only, 2 sinuses with SM elevation and autogenous bone). In 4 animals (2 at each time sequence), one DI placed bilaterally	10, 45 d	
Caneva et al[37]	To assess sequential healing in MSA at sites augmented with either DBBM granules or collagen sponges	20 albino New Zealand rabbits	Bilateral MSA-L. Filled either with xenograft or collagen sponges	7, 14, 21 and 40 d	
Qian et al[35]	To evaluate endo-sinus bone formation after MSA-O with DI without any grafting materials	12 adult male Labrador dogs	MSA-O bilaterally with one implant placed on each site. No grafting performed	4, 8, 24 wk	

	Histological outcomes	Conclusions
	4 d: Sprouts of woven bone from the bony walls towards centre of elevated area. No NBF in close contact with SM. 10 d: Large amount of woven bone sprouting out of the resident bone. 20 d: NBF in continuity with resident bone. NBF appeared to be based on the surrounding resident bone, while no evidence of osteogenetic properties of SM. 30 d: SM lined both on the surface of newly formed bone and connective tissue	Study did not demonstrate any NBF associated with SM but cannot exclude the possibility that SM may have been involved in NBF in later stages of healing or in the presence of filling material
	4 d: No signs of NBF. 10 d: NBF mainly from the base of the sinus floor. Sprouts of newly formed bone in continuity with the parent bone. SM generally lining the provisional matrix, but in some instances directly lining the implant surface. 20 d: NB sprouting from the bony base of the sinus floor and lateral walls and partly in contact with the implant surface. Area below SM appeared reduced in volume and condensed towards the implant apex. No evidence of NBF under SM. 30 d: Implant apex devoid of any bone and only covered by SM. NBF was mainly located at the bony base of the sinus floor and in continuity with the parent bone	SM did not participate in NBF apical to the implants during the early phases of healing, indicating that SM was unable to induce NBF. NBF at implants started from the parent bone of the sinus floor and extended coronally towards the apex of the implants
	15 d: NB sprouting from the resident bone with no NB originating from SM. 30 d: NB appeared to be formed from the parent bone, with no NB originating from SM. 90 d: Osteoclasts on the surface of the NB underneath SM denoting bone resorption	NB sprouting from parent bone and from reflected bony window. SM did not appear to provide a basis for NBF and showed lack of osteogenic potential
	NSSD in % of NB, marrow spaces, connective tissue and xenograft in any of the 3 regions separately and in total between T and C	CM subjacent to SM does not seem to influence healing process, which in turn means that SM was not able to contribute to NBF
	SM elevation only: 10 d: No bone-forming activities could be seen at the elevated SM near the top of the implant. Solitary NBF could be seen in the distal parts of the specimen where SM had been separated from the bone surface. 45 d: Newly formed bone was seen lining SM. Implant apex was in contact with a dense fibrous tissue and finally with the SM only. Perforation of SM: 10 d: Similar morphology as SM elevation only group but without a visible SM. 45 d: A new SM lined the implant. SM touched the tip of the more apical threads and implant apex. Less NBF compared to other groups. SM elevation and bone graft: 10 d: Bone graft particles in granulation tissue to fill the area beneath SM. NBF was seen at the endosteal bone and implant surface as in the other groups. 45 d: Specimens showed a similar morphology to those in the SM elevation only group	NBF after SM elevation with or without additional bone grafts started at the sinus floor and sprouted into the elevated space along the implant surface. The present study does not seem to confirm that SM presents osteoinductive potential in SM elevation procedures
	7 d: NB found at the median wall (13.4 ± 4.4%) and SM region (2.1 ± 3.0%), always in close contact with the pristine bony walls. No NB found in the middle region. 14 d: NBF from the pristine bony walls of the sinus in all regions, reaching the areas that included xenograft granules. 40 d: % NB higher in the medial wall and lower in the SM region (SSD). % connective tissue and biomaterials highest in SM region	NB shown to develop from the pristine bony walls of the sinus and proceeding towards the most peripheral regions. No evidence of NBF from SM
	4 wk: SM collapsed onto the DI apex. Sprouts of woven bone covered by osteoid extending from the parent bone. NB was visible lining the SM and not in contact with DI. No NBF at DI apex. 8 wk: No NB could be seen between DI apex and SM. Inside uppermost threads of implant, small piece of woven bone formed under the SM with no continuity with parent sinus floor or other bone tissues. 24 wk: SM was detected in contact with DI apex. Considerable NB was deposited underneath SM and extending along SM in some cases	NB could hardly be found on the DI apex below the SM at any time point and when present its connection with the parent bone could not be ruled out. Therefore, no clear evidence of the osteogenic potential of SM

Table 4 (cont.) Studies not supporting osteogenic potential of the SM

Study	Purpose	Number and type of animals	Procedure	Timing of sacrifice and biopsy specimen	
Favero et al[39]	To assess the influence of CM covering a perforation of the SM, on the bone formation in the sinus cavity	18 Pelibuey sheep	MSA-L. SM perforation with scissors. CM placed underneath SM (T). No CM at control sites (C). Cavity was filled with BCP (HA: β-TCP 6:4) bilaterally. Access window covered with membrane. Four zones analysed: zone A (cranial portion), zone B (intermediate portion), zone C (floor of the sinus) and zone underneath SM	2, 4, 12 wk	

BCP, biphasic calcium phosphate; β-TCP, beta-tricalcium phosphate; C, control; CM, collagen membrane; DBBM, deproteinised bovine bone material; HA, hyaluronic acid; MSA-L, maxillary sinus augmentation with lateral window; MSA-O, maxillary sinus augmentation with transalveolar technique; NB, new bone; NBF, new bone formation; NSSD, not statistically significantly different; SM, sinus membrane; SS, statistically significantly; SSD, statistically significantly different; T, test.

sinus floor and sprouted into the elevated space, with no observed osteogenic participation from the SM. Interestingly, Jungner et al[17] noted less NBF in the sinus cavity in sites where the SM was perforated.

Caneva et al[37] conducted a study on rabbits in which maxillary sinuses were augmented with bovine-derived xenograft or collagen sponges. They observed NBF from the bony walls during early healing in both groups, with minimal bone formation in the submucosal region, next to the SM.

Two studies on Pelibuey sheep also assessed the influence of collagen membranes (CMs) placed in contact with the SM on the healing outcomes of MSA. Scala et al[38] compared NBF in the submucosal region of sinuses either treated with CMs in contact with the SM or not. They reported no difference in the proportion of NBF between groups at 4 months and concluded that CMs did not seem to influence the healing process and, therefore, that the contribution of the SM to NBF is questionable. Favero et al[39] also compared NBF between maxillary sinuses in which intentionally perforated SMs were either covered with CMs or not. They reported that NBF in the submucosal region was consistently lower than in any other sinus region in both groups. They concluded, similarly to previous studies, that NBF clearly started and progressed from the existing bony boundaries, while patent NBF originating from the SM was not observed.

Animal studies on the local effect of BMPs on the osteogenic capacity of the SM

Nine studies were identified as reporting the effect of BMPs on the osteogenic capacity of the SM (Table 5). The majority reported enhanced NBF in the SM region.

In a rabbit study in which maxillary sinuses were grafted with either hyaluronic acid (HA): β-TCP + rhBMP-2 (T) or HA: β-TCP alone (C), Choi et al[22] noted NBF mostly near the SM in the T group at 2 weeks, whereas in the C group NBF was present mostly in the area adjacent to the existing bone rather than along the SM. However, at 8 weeks both groups had a similar pattern in NBF and the presence of residual graft material. Based on these outcomes, it was assumed that rhBMP-2 stimulates the osteoinductive potential of the SM in the early stages of healing.

In a similar study, Kim et al[40] also noted NBF in close contact with the SM in sites that were grafted with collagenated biphasic calcium phosphate (CBCP) loaded with BMP-2 at 2 weeks post-augmentation. In contrast, minimal NBF was noted adjacent to the host bone in the group that only received CBCP. At 4 weeks, NBF was found in direct contact with the SM at BMP-2 + CBCP sites, whereas in control sites, NBF slightly increased in the SM region.

Hong et al[41], also using biphasic calcium phosphate (BCP) as a carrier for BMP-2, reported that

	Histological outcomes	Conclusions
	2 wk: Minimal NB at both (T) and (C) in all zones. Cavity mainly filled with connective tissue. 4 wk: In both groups, NB in continuity with bony walls of the sinus. In the SM zone, little NB was found only at (T) located nearby the rim of the antrostomy. 12 wk: In SM zone, NB was found at a percentage of 6.7 ± 3.9% (T) and 2.0 ± 2.2% (C) (SSD). NB mainly located close to the rim of the antrostomy. NB on the SM zone was always SS less than the other zones (A, B and C) at all time points. NB 0 (± 0), 3.0 (± 3.3) 6.7 (± 3.9) at (T) and 0.3 (± 0.6), 0 (± 0), 2.0 (± 2.2) at (C) at 2, 4 and 12 wk	NBF clearly started and progressed from the parent wall of the sinus walls towards the centre of the sinus cavity. NBF originating from SM was never observed

NBF was found in direct contact with the SM at 2 weeks post-augmentation in rabbits. In control sites (BCP alone), NBF extended from the host bone, but was never found along or in contact with the SM.

Using a similar MSA model in which porcine bone mineral (PBM) was used as a carrier for BMP-2, Yon et al[21] reported more NBF in the SM region in PBM + BMP-2 sites compared to control sites (11.9 ± 7.8% vs. 5.0 ± 2.3%) at 2 weeks. They concluded that BMP-2 facilitates osteogenesis in regions distant from the antral bony boundaries.

In another rabbit study[42], the addition of N-methyl-2-pyrrolidone (NMP) to rhBMP-2 and BCP increased NBF in regions with lower bone regenerative potential (i.e., the centre of the augmented region and the SM). However, the effects were inconsistent and did not reach statistical significance when compared to bone substitutes alone. Using the same animal model, Yoon et al[43] evaluated a bone patch (Bio-Oss [Geistlich, Wolhusen, Switzerland]: collagen–distilled water) as a carrier for rhBMP-2 in MSA. At 4 weeks, they reported NBF from both the basal bone and the elevated SM.

In a mongrel dog study, Cha et al[18] assessed bovine hydroxyapatite/collagen (BHC) as a carrier for rhBMP-2 and noted that at the BHC + rhBMP-2 sites, NBF was observed in direct contact with the SM at 20 weeks post-augmentation. In contrast, in the BHC-only sites, only a small amount of new bone could be detected in contact with the original sinus floor. Furthermore, NBF was barely observed in proximity to the SM. Similar to previously described studies, the authors concluded that the local delivery of rhBMP-2 improves bone quality in regions of the augmented sinus that are distant from the osteogenic sources of native bone.

Using a rabbit model, Kim et al[20] evaluated the osteogenic potential of BCP + rhBMP-2 in the early healing stages following lateral MSA. They noted that there was 7.86% more NBF in the SM region in sites that received BMP-2 compared to BCP alone. The in vitro component of the same study also showed that when fibroblastic-like cells from the tissue were cultured in osteogenic medium with added BMP, they differentiated into cells that produced mineralising nodules.

Wada et al[44], contrary to previous studies, noted NBF under the SM at 2, 4 and 8 weeks post-augmentation with particulate cancellous bone and marrow (PCBM) or absorbable collagen sponge (ACS) + rhBMP-2. However, the presence of rhBMP-2 did not enhance NBF in contact with the elevated SM, as compared to PCBM.

Discussion

The outcomes of this systematic review, which analysed evidence emanating exclusively from preclinical studies, reveal conflicting evidence regarding

Table 5 Studies on the effects of BMPs on osteogenic potential of the SM

Study	Purpose	Number and type of animals	Procedure	Timing of sacrifice and biopsy specimen	
Choi et al[22]	To establish the osteoinductive effect of rhBMP-2 on HA: β-TCP (3:7) carrier on maxillary sinus and surrounding tissues	18 male New Zealand white rabbits	SMs from 2 animals (4 sinuses) were dissected for in vitro testing and cultured in an osteogenic medium with/without rhBMP-2 for 2 days. Bilateral MSA-L; one sinus grafted with HA: β-TCP + rhBMP-2 (T) and the other with HA: β-TCP alone (C). Window removed and not replaced in the end. 3 regions identified: SM, centre (Ce) and window (W)	2 and 8 wk	
Kim et al[40]	To determine the effectiveness of CBCP as a carrier system for BMP-2 in the early healing stages of MSA	16 male New Zealand white rabbits	Bilateral MSA-L. Test (T): BMP-2-loaded CBCP (BMP group). Control (C): saline-soaked CBCP	2 and 4 wk	
Cha et al[18]	To determine the efficacy of BMP-2 in a BHC carrier to augment bone formation in MSA	8 mongrel dogs	Bilateral MSA-L. Control (C): BHC + Saline T1: BHC + BMP-2 (0.1 mg/ml) T2: BHC + BMP-2 (0.5 mg/ml)	20 wk	
Yon et al[21]	To determine the osteogenic potential of BMP-2 loaded onto a PBM biomaterial in MSA	8 male adult New Zealand White rabbits	Bilateral MSA-L. Sinuses allocated to receive BMP-2/PBM (T) or PBM control (C)	14 d	
Wada et al[44]	To compare bone regeneration of ACS implants impregnated with rhBMP-2 with PCBM in MSA	30 male Japanese white rabbits	Bilateral MSA-L. Sinuses allocated to receive rhBMP- 2/ACS (T) or PCBM (C)	2, 4 and 8 wk	
Hong et al[41]	To assess NBF in MSA during early healing period following grafting with BCP + rhBMP-2	8 New Zealand white rabbits	Bilateral MSA-L microporous BCP with a HA:β-TCP ratio of 3:7 was used as a carrier for rhBMP-2. T: BCP + rhBMP-2 C: BCP alone 3 regions: Window (W), Central (Ce) and Mucosa (M)	2 wk	
Yoon et al[43]	To assess the usefulness of a bone patch as a carrier system for rhBMP-2 in bone regeneration in MSA	5 male New Zealand rabbits	Bilateral MSA-L. Test (T): Mini-implant and bone patch soaked in rhBMP-2. Control (C): Mini-implant and bone patch	4 wk	
Lim et al[42]	To determine whether NMP can decrease the dose of rhBMP-2 needed for bone regeneration in MSA	15 New Zealand White rabbits	3 groups: (1) BMP/NMP (rhBMP-2-coated BCP particles soaked in NMP); (2) BMP (rhBMP-2-coated BCP particles); (3) BCP (BCP particles soaked in saline)	2 wk	

	Histological outcomes	Conclusions
	In vitro: With rhBMP-2, increased expression of early osteoblasts with significant upregulation of RUNX2, type I collagen, alkaline phosphatase and osteopontin. Expression levels of late osteoblasts like osteocalcin not significantly changed. Histology: 2 wk (T): NB mostly near SM in the rhBMP-2 group. No evidence of NB in window and central regions. 2 wk (C): NB all around regenerated area but more extensively at the area adjacent to the existing alveolar bone rather than along SM. NB sprouted out from the lateral bone inside the augmented sinuses. 8 wk: T and C had similar pattern of bone formation and appearance of residual material with newly formed woven bone replaced by lamellar bone. % NB at C: W region (2 wk vs. 8 wk): 7.27 ± 5.09 vs. 26.81 ± 10.42 SM region (2 wk vs. 8 wk): 15.58 ± 4.33 vs. 28.17 ± 7.94 Ce region (2 vs. 8 wk): 7.41 ± 5.21 vs. 22.14 ± 7.83 % NB at T: W region (2 wk vs. 8 wk): 0.06 ± 0.18 vs. 19.97 ± 5.58 SM region (2 wk vs. 8 wk): 20.86 ± 12.05 vs. 29.33 ± 12.05 Ce region (2 wk vs. 8 wk): 0 vs. 17.69 ± 6.83	Different healing patterns along SM in rhBMP-2 treated and control sites. It can be assumed that rhBMP-2 might provoke osteogenic differentiation of progenitor cells of SM in the early healing phase
	% NBF at SM region was SS greater in T vs. C. 2 wk (C): Small amount of NB adjacent to pristine bone. 2 wk (T): NB observed along the outer surface of CBCP, close to the parent bone wall and SM. 4 wk (C): NB increased in the window and SM region; sparsely observed in the central portion of the augmented area. 4 wk (T): Considerable amount of NB evenly distributed throughout entire augmented sinus. NB was found in direct contact along SM. At the 2-wk healing point, only the SM region showed a SSD in % NB between T and C	Osteoinductive potential of SM is provoked in the early stages of healing with BMP-2
	C: Most of the bovine hydroxyapatite particles were encapsulated by fibroblastic cells with smooth borders in the whole augmented area, and only a small amount of NB could be detected in the original sinus floor. T: NB observed in direct contact with SM. NBF was significantly greater in all of the BMP-2–treated groups than in C, not only in the central portion of augmented area close to the basal bone but also in the area near SM	Improved bone qualities at the peripheral portion of augmented sinus distant from osteogenic sources in all BMP-2–treated sites, whereas NBF was barely observed underneath SM in the control group
	NB observed in the window (W), centre (Ce) and SM regions. C: NB found mainly adjacent to resident bone in W, whereas little NB in Ce and SM regions. NB in Ce and SM regions was significantly greater in T than in C (Ce: 14.2 ± 7.7% vs. 5.5 ± 5.5; SM: 11.9 ± 7.8% vs. 5.0 ± 2.3%), whereas NB in W region did not differ significantly between groups. In T, the area of NB was significantly greater in W than in the other two regions. In C, NB was significantly greater in W than in the other two regions	BMP-2 might facilitate osteogenesis in regions distant from the resident bone such as Ce and SM regions (osteogenic stimulation of SM by BMP-2)
	T: At 4 wk, the trabeculae were more mature than at 2 wk and observed under intact SM. C: Immature new cortex was observed at the lateral osteotomy site and under SM. At 8 wk, cortical bone formation was observed under SM and at lateral sinus wall	Immature new cortex observed at the lateral osteotomy site and under elevated SM earlier at the PCBM sites than BMP sites
	T: NB was rarely found in W and Ce whereas it was found in the M region in direct contact with the SM. C: NB extended from the lateral wall of the pristine bone, however no NB along or in contact with SM	In test, NB in direct contact with SM, suggesting the presence of osteoprogenitor cells in SM and osteoinductive potential of rhBMP-2 along SM
	SM in contact with bone particles and NB. Most of the NB sprouted from the basal bone and grew along the implant surface. C: NB in direct contact with bone substitute particles was rarely observed. T: NB grew to the SM in tight contact with DI and formed a bridge with remaining graft particles	rhBMP-2 facilitated NBF on SM
	Bone formation occurred predominantly near native bone walls (i.e., bony window and lateral walls) in all groups. NB started from the margins of the bony window, the lateral sinus walls and SM. NBF in the centre of the sinus and in proximity to SM appeared to be greater in BMP/NMP than in BMP and BCP. However, the increase in NB with the application of NMP did not reach statistical significance	Using NMP as an adjunct to rhBMP-2–coated BCP increased bone regeneration in the augmented sinus, specifically in the centre of the augmentation and the region close to SM (NSSD)

Table 5 (cont.) Studies on the effects of BMPs on osteogenic potential of the SM

Study	Purpose	Number and type of animals	Procedure	Timing of sacrifice and biopsy specimen	
Kim et al[20]	To evaluate the osteogenic potential of BCP coated with a rhBMP-2 dose of 0.015 mg in the early stages of healing	8 New Zealand white rabbits	Bilateral MSA-L. Window removed and not replaced. Test (T): BCP lyophilised with rhBMP-2; Control (C): BCP soaked with saline. SM tissue collected during MSA. Fibroblastic cells (T) cultured in osteogenic induction medium and BMP added at various concentrations for 4 wk. Fibroblastic cells (C) cultured in osteogenic induction medium only	2 wk	

ACS, absorbable collagen sponge; ALP, alkaline phosphatase; BCP, biphasic calcium phosphate; BHC, bovine hydroxyapatite/collagen; BMP, bone morphogenetic protein; β-TCP, beta-tricalcium phosphate; C, control; CBCP, collagenated biphasic calcium phosphate; Ce, centre; FV, fibrovascular tissue; NB, new bone; NMP, N-methyl-2-pyrrolidone; NSSD, not statistically significantly different; PBM, porcine bone mineral; PCBM, particulate cancellous bone and marrow; RG, residual graft; rhBMP, recombinant human bone morphogenic protein; SM, sinus membrane; SS, statistically significantly; statistically significantly different; T, test; W, window.

the contribution of the SM to NBF in MSA. A consistent histological finding across multiple studies was NBF initiating from the sinus floor and sprouting into the augmented region. This corroborates the osteogenic potential of the bony boundaries of the maxillary sinus, but a point of controversy was whether the SM exhibits such potential. The main variable that was assessed to evaluate the effect of the SM in NBF was the presence of NBF in proximity to the membrane after a variable healing period. The inclusion of histological outcomes at later stages of healing (6 months) may be misleading, however, as the NBF in proximity to the SM at that stage could derive from the surrounding bony walls and not exclusively from the SM itself. Nevertheless, a decision was made to include longer healing periods for comprehensiveness, as currently there is no solid evidence supporting or refuting this hypothesis.

As previously mentioned, histological outcomes of NBF in the submucosal area were only reported in preclinical studies. Unfortunately, the identified human studies that reported histological/histomorphometrical outcomes involved the analysis of bone core biopsies without distinguishing between the various areas of the sinus (i.e. close to the host bone, central sinus region, submucosal area). Thus, the absence of a separate analysis of NBF in the submucosal area excluded such studies as they could not provide any assessment of the osteogenic potential of the SM. Only one human clinical study[45] that investigated the osteogenic potential of the SM through separate analysis of the submucosal area was identified, but this was done through radiographic evaluation, which was not compatible with the inclusion criteria. In this study, NBF was evaluated after MSA using radiolucent collagenous sponges and DICOM data evaluation to monitor the calcification process at 4 and 7 months. An even and circular centripetal calcification under the SM and antral floor was noted at 4 months which, according to the authors, outlined the key role of the SM as a carrier of bone reformation after MSA procedures[45].

Most of the in vitro studies analysed agreed that the SM contains pluripotential mesenchymal stem cells and reported that isolating and culturing these cells in an osteogenic medium results in extracellular mineral formation, as well as expression of ALP and osteocalcin markers[8-11,13,15]. Interestingly, Guo et al[14] noted that mesenchymal stem cells isolated from the SM, apart from their potential for osteogenic differentiation, are also able to differentiate into other cell types, including adipocytes and chondrocytes, depending on the culture medium. It is worth noting that if these cells were cultured in non-osteogenic medium, no osteogenic differentiation was noted, which led Yun et al[12] to suggest that SM stem cells may have no inherent osteogenic potential, but need the presence of an osteoinductive co-factor in order to participate in mineralised tissue formation. This

	Histological outcomes	Conclusions
	T: NB observed along SM, well integrated into FV and RG. C: Greater NBF evident at the periphery of the defect margin of the windows. SM area composed mainly of FV. NSSD in NBF between T and C. 7.86% more NB in the SM area in T vs. C (possibly due to rhBMP-2–induced enhancement in the osteogenic potential of SM). In C, 50.1% more NB was found in the window area than in the SM area. In both T and C, SS greater NB in the window vs. centre and membrane vs. centre. In vitro: Fibroblast cells differentiated into cells to produce mineralised nodules. Increase in the number of mineralised nodules in the BMP treatment groups. Increase in ALP production (BMP concentration–dependent)	In C, NBF started from the remaining bone, from the sinus walls and the septa. No apparent osteogenic potential or remarkable NBF in the early healing period when using a low dose of rhBMP-2, although in vitro analysis showed enhanced osteogenic potential of SM with rhBMP-2

notion is partially supported by the outcomes of different animal studies in which an osteoinductive factor was used. More specifically, the use of BMPs in MSA procedures seems to significantly affect the bone-forming properties of the SM and the timing of NBF. The majority of the included studies reported that in the presence of BMPs, a greater proportion of NBF was noted in the sinus region close to the SM compared to non–BMP-treated sites. NBF was particularly robust in the early healing stages, which may suggest that the presence of BMPs in proximity to the SM may enhance the differentiation of pluripotent mesenchymal stem cells into an osteogenic lineage, resulting in enhanced NBF.

In contrast, in the animal studies in which no BMPs were used, the findings pertaining to the bone-forming abilities of the SM were contradictory. Specifically, from the 16 studies that did not involve the use of BMPs, 8 reported outcomes supportive of the notion that the SM has bone-forming properties. Palma et al[3] and Cricchio et al[27] reported NBF in contact with the SM after a 6-month healing period following MSA using dental implants or a space-making device to hold the membrane elevated and coagulum to fill the space. This finding, according to the authors, confirmed the osteoinductive potential of the SM. In contrast, Qian et al[35] found hardly any NBF around the apex of dental implants and, when present, it appeared to stem from the native bone. Similarly, Scala et al[16,34], in two studies using the same animal model and a similar surgical protocol as in the previously described studies[3,27], did not find any NBF lining the SM at biopsies harvested at 4, 10, 20 and 30 days after MSA, thus questioning the bone-forming properties of the SM. However, they noted that the collapse of the SM into the implant apex could be a bone-forming limiting factor and did not exclude the possibility that the SM could participate in NBF in the presence of graft materials assisting with space maintenance. On that note, a number of studies assessed whether the presence of bone grafting materials affects the pattern of bone healing in the region close to the SM. Jungner et al[17] reported similar healing patterns in sinuses with or without additional bone grafts, indicating that NBF started primarily at the sinus floor with no evidence of osteoinductive potential from the SM. Similarly, Caneva et al[37], in a study on sequential healing at sites augmented with xenograft in comparison to collagen sponges, noted that NBF developed from sinus bone walls but that there was no evidence of NBF from the SM. In two control studies aimed at evaluating the healing process after SM elevation with or without bone grafting, Sohn et al[4,31] reported that NBF started from the elevated SM and progressed to the central region of elevated subantral space as healing time increased. They noted the presence of a higher proportion of NBF on the SM than at the central area of the

augmented region, irrespective of the presence of graft material. According to the authors, this was indicative of the osteoinductive properties of the SM. Thus, based on the above findings, the presence or absence of bone grafting materials along with their associated space maintenance properties does not seem to be a factor affecting the bone-forming properties, or lack thereof, of the SM. Rong et al[30] and Scala et al[38] also reported contradictory outcomes when assessing the osteogenic capacity of the SM through the placement of cell-occlusive barriers between the bone grafting material and the SM. Specifically, Rong et al[30] reported NBF at 3 months around particles adjacent to the SM when no barrier was placed, whereas in the presence of a thin titanium mesh interposed between the bone graft and the SM, most graft particles were enveloped in loose connective tissue, thus supporting the osteogenic capability of the SM. Scala et al[38] reported a similar percentage of NBF at 4 months, irrespective of whether a collagen barrier was placed in between a particulate xenograft and the SM, and concluded that the SM does not contribute to NBF.

Extrapolation of healing outcomes following MSA procedures performed in animal studies to humans is not without limitations. Almost half of the included animal studies used rabbits, which are considered to be an appropriate model for simulating a human sinus due to anatomical similarities[46]. However, some important differences should be taken into account, including the bone remodelling period, which is known to be shorter in rabbits, as well as the air pressure over the graft area, which has been reported to be larger in rabbits than in humans[47]. Similarly, for studies using dogs as an animal model, the differences in average SM thickness, which is between 0.6 mm and 1.4 mm for dogs and between 0.3 mm and 0.8 mm for humans, may also affect the sinus healing patterns and the likelihood of a perforation[48]. In summary, because of the effect that these differences may have on the function and properties of the SM in humans as compared to animal models, no definite conclusions on the bone-forming properties of the human SM can be drawn based on animal models.

Conclusion

Within the limitations of this review, it can be concluded that the SM contains pluripotential cells with the capacity to differentiate and stimulate NBF. However, the findings from the studies selected in this systematic review do not consistently support that the SM significantly contributes to NBF following MSA procedures. In fact, NBF in proximity to the SM after MSA procedures was not a consistent finding in the majority of the preclinical studies selected in this review and, when observed, it was lower than in areas in proximity to the antral bony boundaries. Local delivery of BMP-2 seems to upregulate the osteogenic potential of SM cells to enhance NBF, particularly in the early stages of healing.

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Panagiotis Dragonas

Panagiotis Dragonas, DDS, MS
Assistant Professor, Department of Periodontics, School of Dentistry, Louisiana State University Health Sciences Center New Orleans, New Orleans, FL, USA

Theodoros Katsaros, DDS, MS
Assistant Professor, Department of Periodontics, College of Dentistry, University of Iowa, Iowa City, IA, USA

Julie Schiavo, MLIS, AHIP
Assistant Director, Department of Libraries, Louisiana State University Health Sciences Center New Orleans, New Orleans, FL, USA

Pablo Galindo-Moreno, DDS, PhD
Professor, Department of Oral Surgery and Implant Dentistry, School of Dentistry, University of Granada, Granada, Spain

Gustavo Avila-Ortiz, DDS, MS, PhD
Professor, Department of Periodontics, College of Dentistry, University of Iowa, Iowa City, IA, USA

Correspondence to:
Dr Panagiotis Dragonas, School of Dentistry, Department of Periodontics, 1100 Florida Avenue, Box 138, New Orleans, LA, USA, 70119. Email: pdrag1@lsuhsc.edu