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Title page

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Running Title : Materials to enhance ACL Tendon Graft To Bone Healing

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Manuscript

Abstract

Purpose: To perform a systematic literature review to analyze the results of the *in vivo* animal models and strategies that use osteoinductive materials to enhance the tendon graft - bone interface for anterior cruciate ligament reconstruction (ACLR). **Methods:** Following the PRISMA guidelines, the PubMed, Embase and Web of Science databases were searched. The inclusion criteria were studies of *in vivo* animal models of ACLR using a material to enhance tendon graft – bone interface healing and reporting at least the histological results at the interface, along with radiological and biomechanical data. Studies without control group or with another tendon-bone healing model were excluded. Methodological quality was assessed with the ARRIVE guidelines. . **Results:** Twenty-seven studies met the inclusion criteria. Rabbit was the main animal model of ACLR, along with sheep and dog models. ACLR procedures varied widely between studies.. The main promising strategies and materials were wrapping the material around the graft, with a collagen scaffold loaded with an osteoinductive molecule (mostly BMPs). The second strategy consisted on injecting the material at the tendon – bone interface; calcium phosphate cement or a derivative were the most used material. Finally, using osteoinductive fixation devices was the third strategy; magnesium-based interference screws seemed to show most favorable results.. **Conclusions:** The studies retained had major methodological flaws that limit the scope of these conclusions. However, based on histological, biomechanical and radiological analyses, the most promising materials were a collagen scaffold loaded with an osteoinductive molecule and wrapped around the graft, calcium phosphate cement injected in the bone tunnel and use a magnesium-based fixation device. **Clinical relevance:** *In vivo* animal models have identified several promising strategies and materials to optimize the tendon - bone interface after ACL reconstruction, but standardized and reproducible assessments are needed before these strategies can be adopted clinically.

Introduction

Anterior cruciate ligament (ACL) injury is one of the most common knee injuries in teenagers and young adults. 200,000 to 400,000 ACL tears are diagnosed and 175,000 reconstruction procedures are done each year in the United States[1,2]. High-quality tendon-bone anchoring allows for return to pivot sports from 9 to 12 months after ACL reconstruction (ACLR). According to Grana *et al*., the ACL tendon graft - bone interface heals by the formation of fibrous tissue with an indirect insertion to bone (Sharpey fibers), combined with bone growth[3]. However, this healing does not reproduce the ACL's original direct insertion, in which the ligament fibers meet the bone perpendicularly, then successively become fibrocartilage, mineralized fibrocartilage and finally bone [4,5]. In all, healing at the tendon – bone interface after ACLR requires about 12 weeks[6] before the tendon graft itself becomes the weakest point of the construct altough this shift appears to begin around the 6th week Different strategies have been introduced to accelerate and enhance ACL tendon graft to bone interface healing[7], [8],[9], . Among them, insertion of osteoinductive materials, or drug delivery systems at the tendon graft – bone interface, seems to be a very promising strategy. It appears cost-effective and could easily be integrated into ACLR. However, the literature remains unclear about which material or drug delivery system effectively improves the healing of tendon graft to bone interface. *In vivo* assessment of the materials used in this strategy through animal models of ACLR are an useful tool to compare the tendon graft to bone healing.

To our knowledge, no study has analyzed and compared the healing of tendon graft – bone interface in animal models, *in vivo*, based on a potential osteoinductive material applied at this interface. Thus, the purpose of this study was to perform a systematic literature review to analyze the results of the *in vivo* animal models and strategies that use osteoinductive materials to enhance the tendon graft - bone interface for anterior cruciate ligament

reconstruction (ACLR). We hypothesized that this systematic review could summarize the main results of these strategies and identify which material provide favorable results.

Materials and Methods

Search strategy

A search was performed of the PubMed (Medline), Embase, and Web of Science databases in May 2021 while following the PRISMA (Preferred Reporting Items for Systemic Reviews and Meta-Analysis) guidelines[10]. A combination of terms compatible with the systematic literature review were used: “tendon-bone” or “tendon graft-bone” combined with the terms, “histology”, “tissue engineering”, “in vivo”, “animal model”, “scaffold”, “interface”, “healing”, “repair”, “ACL reconstruction”, “knee” or “knees”.(Appendix 1). The references of the studies included in this systematic literature review were also checked to identify other pertinent references that should be added. Study selection and data extraction were done independently by two of the study’s authors (M.S. and F.C.). Any disagreement was resolved by discussion between these two authors or by asking a third author for input.

Inclusion and exclusion criteria

Studies were eligible for inclusion if they met the following criteria: *in vivo* animal models of ACLR using materials and drug delivery scaffolds. The materials could be either synthetic or natural. All animal models were included if the subjects underwent ACL tendon graft reconstruction after complete ACL excision followed by an analysis of the tendon- bone interface. At a minimum, a histological analysis of the tendon graft – bone interface was required, possibly associated with other analysis (imaging and/or biomechanics).

Studies were excluded for the following reasons: language other than French or English, other literature reviews, comments, letters to editor, book chapters, conference abstracts, no biomaterials used, cadaver study, studies about other joints or ACL reconstruction with bone-to-bone anchoring, studies of the repair or augmentation of the native ACL, use of synthetic graft or xenograft, use of the bone tissue extracted from animal tissue, studies done in humans (since this literature was solely focused on animal models), studies without control groups and

studies involving an extra-articular animal model of tendon-bone healing. Indeed, one of the accepted limitations of extra-articular models is the lack of exposure to articular fluid, which can be responsible for tunnel widening mediated by pro-inflammatory cytokines, but also the inability to reproduce mechanical loads to which the grafts are subjected, such as the windshield-wiper effect or bungee effect[11–14]. Lastly, this systemic review excluded studies where drugs or osteoinductive peptides were used without a scaffold for controlled release , studies in which gene therapy was used on the cells in the graft or studies involving stem cells (cultured and/or genetically modified).

Selection of studies

The articles were initially screened by reading the title and abstract. All the studies that met the eligibility criteria were included in the analysis of whole text articles retrieved from the database.

Evaluation of methodological quality

This was done using a checklist based on the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments)[15]. It evaluates the reliability and reproducibility of *in vivo* animal studies according to 10 criteria (The Essential Set, maximal score : 10/10) considered as essential and indispensable for a study of high methodological quality. Eleven additional items (Recommended Set, maximal score: 11/11) were also evaluated to refine the evaluation of the methodology. In this review, the studies retained were evaluated using both sets of the guideline. Each item was scored as 0, 0.5 or 1 (0.5 corresponded to partial validation when certain subitems were missing, maximal score : 21/21). This evaluation was also done independently by two of the study authors and any disagreement resolved by discussion.

Data extraction

The data extracted pertained to the animal species, number of animals and the experimental model: type of graft, graft fixation method, material(s) used and if any drugs were added. Other items also being captured were the controls , follow-up time, methods used to analyze the tendon graft – bone interface and the results at each time

point analyzed. For the histological analysis we extracted available data involving qualitative observations of the healing: presence or not of Sharpey-fibers, fibrocartilage and new bone. Quantitative data were also extracted (histomorphometry, quantitative immunofluorescence). When a biomechanical analysis had been carried out, we extracted the type and load applied to the construct, the ultimate failure load (UFL) , the stiffness and share of failures of at the interface by pull out from the tunnel. Finally, when a radiological analysis had been performed, data about the type of imaging, qualitative and quantitative analysis were extracted. .

Results

Selection of studies

A total of 1712 studies were identified after removing duplicates. After screening the titles and abstracts, 1216 were excluded as they met one or more of the exclusion criteria. Finally, 27 studies were retained for full text analysis, including 4 identified in reference lists of included studies (Figure 1).

Methodological quality (risk of bias)

The mean score based on the ARRIVE Essential 10 checklist was 7.9 ± 0.9 (min-max 6.5-9) and 14.7 ± 1.3 (min-max 12,5-17) based on the Recommended Set (Table 1). The essential items that were often missing was the *a priori* calculation of the number of animals needed for statistical comparison, which was only done in 6 (22%) studies[16–21] and randomization, which was reported in 12 (4%) studies, of which 2 (7%) entirely met this ARRIVE criteria [22,23]. Blinded assessment of the study's endpoints was only done in 8 (29%) studies [21–28].

Study characteristics

Animal models

The most frequently used animal model was the rabbit (18/27), followed by sheep (5/27) and dog (4/28). All studies used an open ACL excision-reconstruction model. Except for two studies using allografts[20,24], autografts

consisted of, *semitendinosus* (10/27) or *flexor digitorum* (7/27), and less often the *long digital extensor* (6/27). All of the studies reported the diameter of the bone tunnels made, although the diameter varied even within the same animal species. For rabbits, the diameter varied widely between 1.2 and 3.2 mm. Only 10/27 studies reported the diameter of the grafts used and inserted in the bone tunnels[24,26–34]. Finally, the fixation method of the graft varied widely between studies. It consisted of suturing it to the periosteum and/or adjacent soft tissues adjacent (17/27 studies), suspension suturing (5/27/) or using of fixation devices (9/27) (Table 2).

ACL tendon graft to bone interface enhancing strategies and materials

The "wrap-around" strategy was used in 9/27 (33%) studies. The material was wrapped around the intra-tunnel portion of the prepared graft, which was subsequently inserted into the bone tunnels. These materials consisted of natural polymers such as collagen or gelatin (6/28), chitosan[35], , used in the form of a sponge or hydrogels [16,21,22,34,36,37] or synthetic ones such as PCL used in combination[35,38] in the form of electrospun nanofibers The material served as a reservoir for a potential osteoinductive molecule and was compared to a control group that received a wrapped graft, without the molecule.

The second strategy consisted of injecting the material between the graft and bone tunnel (10/27). The injected material could be a calcium phosphate cement (7/10), or glues made of collagen[23], fibrin[27] andmagnesium[19]. All studies in this strategy included an ACLR without the material injected as control groups.

The third strategy consisted of giving osteoinductive properties to the fixation device itself (5/27). The most commonly used device was the interference screw (4/5), made of pure or a magnesium-based alloy[25,39,40] or polycarbonate[17].

These materials could also be used as drug-release systems at the tendon graft – bone interface (13/27 studies). BMPs were studied the most (7/27)[16,30,33–36,41] along with other potential osteoinductive factors such as G-CSF[22], TGF-B1[27], FGF[23], SDF[35], OPG[34] and simvastatin[37].

Mutsuzaki *et al*. used a particular technique in which the graft was submerged in a calcium solution then phosphorous solution before being inserted in the tunnels[20,26,28,31]. (Table 2).

Outcomes of ACL tendon graft to bone interface evaluation

Histology

All the studies reported at least a qualitative description of the interface. Presence or absence of fibrocartilage, Sharpey fibers and/or new bone was not routinely evaluated and compared in these descriptions. Findings were sometimes summed up with histologic scores (5/27) (Appendix 2). Quantitative data were found in 16 studies and the main comparison was about new bone formation (Table 3). At 2-3 weeks after ACLR, materials wrapped around the graft or injected in the tunnel and loaded with BMP[36,41], OPG[18] and simvastatin[37] significantly accelerated new bone formation compared to the material alone. At 8 weeks, a wrapped collagen scaffold loaded with OPG and BMP2[33] and calcium-phosphate cement loaded with BMP[41] promoted superior bone formation compared to controls.

Biomechanics

A biomechanical analysis was carried out in 24/27 studies: except for one study[26], all the tests applied a tensile load parallel to the axis of the graft. Six of 10 studies that reported results at 2-3 weeks found significantly higher UFL with wrapped scaffolds associated with BMP[16,36] or simvastatin[37], injected calcium-phosphate cement[29,42], fibrin associated with TGF-B1[27], compared with control groups. However, the mechanism of failure was always a pull-out from tunnel. Seventeen studies reported results between 4 and 6 weeks and nine of these studies reported higher UFL compared to control groups. Two of three studies reported no difference in UFL in ACLR with calcium phosphate cement + BMP compared to control groups [18,41]. Several studies described a shift in the weak point from tunnel to midsubstance failure but only one study reported a statistically significant difference at 6 weeks[32]. Nine studies reported results at 8 weeks and it was at this time point that electrospun membranes or injected calcium phosphate cement associated with osteoinductive molecules produced higher UFL compared to controls (results non-significant before this time point)[18,35,38,41]. Wrapped or injected collagen scaffolds associated with either BMP[16],PRP[21] and FGF[23] resulted in higher UFL compared to controls without any difference in the failure site at 8 weeks. At 12 weeks, two of the three studies featuring the injection strategy still reported significantly higher UFL[23,32]. The studies included in the fixation strategy rarely reported biomechanical results before 12 weeks and these were not significant. After this time point, magnesium-based fixation devices produced higher UFL in three of four studies compared to traditional materials [30,39,40] (Table 4).

Imaging

Fifteen studies (54%) reported a radiological analysis and among them 10 reported quantitative data. Micro-CT was the most frequently used modality (9 studies). New bone formation was most often featured; it was

determined based on bone tunnel area or diameter and bone volume or density. The wrap-around strategy (4 studies) reported a significant reduction in bone tunnel area with gelatin + G-CSF[22] or simvastatin[37] at 4-6 weeks. For the injection strategy (5 studies), Calcium-phosphate cement with BMP resulted in smaller tunnel diameters from 2-3 weeks[33,41] to 8 weeks[41]. Brushite calcium phosphate cement increase bone formation at 6 and 12 weeks[32]. Finally, interference screws made of magnesium alloys increase bone formation in femur tunnels at 6 weeks[39,40]. After this time point, Mg-based screws progressively degraded and were replaced by new bone (Table 5).

Discussion

This systematic literature review identified three main strategies and several materials to enhance ACL tendon graft to bone interface healing in animal models, *in vivo*.

Animal models

The most frequent animal model was the rabbit model but larger animals(ovine and dogs) were also used. The diameter of bone tunnels varied widely, even in the same animal model. Moreover, , the diameter of the graft itself was only disclosed in 10/27 studies. This data should always be reported to allow for comparison between graft sizes, especially because this systematic review identified more than three types of grafts used for ACLR. For example, studies that adopted the graft wrapping strategy did not always provide the dimensions of the material enveloping the graft, particularly its thickness, except for two studies[21,22]. This piece of information is highly relevant since this thickness is added to the graft's diameter and must be taken into account when selecting which size of bone tunnel to make. Parameters of ACLR in animal models would be standardized or at least reported in greater detail to improve comparability and reproducibility between studies.

Evaluation of the tendon graft to bone healing

All studies provided at least an analysis between 6 and 12 weeks, except for the studies focused on resorption of the fixation device (up to 52 weeks)[17]The histological analyses were often qualitative. Furthermore, all tissues of interest (fibrocartilage, sharpey fibers and new bone) within the interface were not regularly reported. While these results could be summed up with a histological score, six different scores were used[17,43–47] Quantitative histological analysis should be done more often to allow comparison between studies (16/27 studies).. The radiological analysis

was quantitative in 10/27 studies. The main criteria were the size of the bone tunnel or the new bone formation, evaluated through various micro-CT measurements. Comparison between studies would be allowed if these criteria were more consistent.

Most of the studies included a biomechanical analysis of the interface by a pull-out testing of the ACLR that reflected the strength of the interface. The heterogeneity came for the pull out testing speed applied to the grafts: 0.5 mm/min to more than 40 mm/sec. Except for one study[26], the biomechanical analyses did not reproduce the true cyclic loading to which grafts are subjected to; however, no study incorporated postoperative immobilization, which theoretically exposed the operated knees to the same loads as the native knee.

This systematic review identified the need for any qualitative histological analysis to report at least presence or absence of fibrocartilage, Sharpey fibers and new bone at the interface, possibly by using a histologic score. All studies should include quantitative measurements such as histomorphometry or quantitative immunofluorescence to support qualitative observations. If radiological comparison is provided, quantitative data on new bone formation and/or tunnel size should be reported through micro-CT measurements. Finally, biomechanical analysis seemed to reflect the strength of the interface and so the relevance of the material tested. A harmonized tensile load should be defined for a specific animal model in order to compare results (ultimate load to failure, tunnel failure, etc.).

Wrap-around strategy

This strategy used a material in a form that could be loaded with a potential osteoinductive molecule, making this material act like a reservoir, and was compared to the same material alone. Collagen or gelatin was the most frequently studied material. Results suggested that loading BMP into this scaffold could promote early bone formation[36] and increase the ultimate failure load of the ACLR from 2-3[16,36] weeks to 8 weeks[16,34]. Other molecules studied had inconsistent results from a biomechanical viewpoint but seemed to limit bone tunnel enlargement during healing of the interface[22,37] based on histological and radiological analysis. Overly fast degradation of sponges or hydrogels may have led to the recent development of electrospun nanofiber membranes made of synthetic polymers whose resorption rate could be controlled. Two studies reported results of PCL electrospun membranes, loaded with BMP and SDF[35] or hydroxyapatite[38]. These loaded membranes increased the UFL at 12 weeks compared to membranes alone, but not before this time point. This suggests that the electrospun membranes provided sustained release of the molecule of interest.

Injection strategy

The material injected was mainly a calcium phosphate cement (70%) possibly loaded with BMP or other molecules (3 studies). Higher UFL was found with this material or its derivative early on [29,42], and at later time points [29,32]. Interestingly, adding an osteoinductive molecule didn't seem to clearly improve the pull-out strength with this material as comparisons were non-significant for two of the three studies[18,41]. However, histological and radiological analysis suggested that BMP-loaded cement helped new bone ingrowth into bone tunnel[33,41]. Analysis of the degradation or repopulation in cases of calcium phosphate matrix, or confirmation of no release into the joint were not reported regularly, which brings into question the applicability of these potentially osteoinductive materials during arthroscopic procedures. Also, it could be difficult to confirm circumferential deposition into the tunnel when injecting the material once the graft is in place; certain studies got around this by injecting the material into the tunnel before inserting the graft. A collagen scaffold loaded with FGF was also injected and produced higher UFL at 4 and 12 weeks[23]. Finally, a glue made of magnesium, calcium an phosphate was injected in tunnels and increased the UFL at 6 weeks along with smaller interface width in histomorphometry and higher bone volume on radiological analysis (non-significant at 3 weeks)[19].

Fixation strategy

Adding osteoinductive properties to fixation devices was mainly studied with interference screws. Currently made of resorbable polymer, these screws have similar biomechanical properties to titanium screws while limiting their drawbacks[48]. However, they do not have osteoinductive properties and can remain intact in the tunnel for more than 1 year[49,50]. Magnesium was mostly used in this strategy (4/5 studies). Pure magnesium or magnesium-based alloys used for interference screws or crosspin fixation resulted in higher UFL compared to standard devices at 12 weeks in three of four studies but not before this time point. Magnesium may accelerate fibrocartilage formation [25], mesenchymal stem cell recruitment and fracture healing *in vivo* [40]. While Cheng *et al.* used an interference screw made of 99.98% magnesium, several studies have found that pure magnesium has poor mechanical strength and will corrode rapidly. These two problems appear to have been resolved by the development of alloys combining magnesium with zinc, manganese[40], calcium[51] or strontium[39]. The speed of repopulation of the interference screw by bone tissue was much faster than with synthetic polymers and may prevent bone tunnel widening[39,40]. However, these studies analyzed fixation within a femoral tunnel while the tibial tunnel is more likely to be affected by tunnel widening[39,40].

. The studies by Mutsuzaki et al. adopted a unique strategy: the graft was submerged in a calcium solution then a phosphorus one. In a prospective randomized, controlled study in humans with 2 years' follow-up, this procedure was found to be safe and provided functional improvement relative to preoperative levels and comparable to that of conventional ACL reconstruction[52].

Limitations

This systematic review had some limitations. The analysis of the methodology using the ARRIVE checklist identified certain major deficiencies leading to possible bias: incomplete information about randomization (15/27 studies), no blinded evaluation of the results (19/27 studies) and *a priori* calculation of the number of subjects needed (21/27 studies). The absence of these essential elements limits the scope of the results found and our ability to compare studies. Second, animal models of ACLR varied but the rabbit model was the most frequent. Unfortunately, the ACLRs were not comparable as they had different grafts, different fixations and different tunnel sizes, thus further limiting the scope of the results. Third, the type and characteristics of the histological, radiological and biomechanical analysis methods varied widely, which made it impossible to compare results for ACL tendon graft to bone interface improvement between strategies or within a same strategy but with different materials. Fourth, this systematic review did not consider other ACL tendon graft to bone interface improvement strategies reported in the literature such as cell therapy[7], gene transfection using viral vectors[8], optimization of the reloading protocols[47] or the use of low-intensity pulsed ultrasound therapy[53]

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Conclusion

The studies retained had major methodological flaws that limit the scope of these conclusions. However, based on histological, biomechanical and radiological analyses, the most promising materials were a collagen scaffold loaded with an osteoinductive molecule and wrapped around the graft, calcium phosphate cement injected in the bone tunnel and use a magnesium-based fixation device.

Figure legends:

Figure 1: PRISMA flow diagram of this systematic review

Acknowledgement: *The authors are grateful to Joanne Archambault, PhD for English language assistance.*

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Table 1: Evaluation of the methodological quality of the included studies

Year/Authors	ARRIVE Essential 10 (1 = total, 0.5 = partial, 0 = none)	Essential 10 Score (/10)	ARRIVE Recommended Set (11 items) (1 = total, 0.5 = partial, 0 = none)	Total Score (/21)
2001/ Anderson et al.[16]	1/1/1/0.5/0/1/1/0/1/1	**7.5**	1/1/1/1/0.5/0/1/1/0/0/0.5	**14.5/21**
2004/ Tien et al.[42]	1/0.5/1/0/0/1/1/1/1/1	**7.5**	1/1/1/0/0.5/0/0.5/1/0/0/1	**13.5/21**
2004/Mihelic et al.[36]	1/0.5/1/0.5/0/1/0.5/1/1/1	**7.5**	1/1/1/0.5/0/0/0.5/1/0/0/1	**13.5/21**
2004/Mutsuzaki et al.[31]	1/0.5/1/0/0/1/0.5/0.5/1/1	**7**	1/1/1/1/0/0/0.5/0.5/0/0/0.5	**12.5/21**
2005/ Yamazaki et al.[27]	1/0.5/1/0/0.5/1/1/0.5/1/1/	**7.5**	1/1/1/0.5/0.5/0/1/1/0.5/0/0.5	**14.5/21**
2007 / Huangfu et al.[29]	1/0.5/1/0.5/0/0.5/0/1/1/1	**6.5**	1/1/1/1/1/0/1/1/0/0/0.5	**14/21**
2007/Walsh et al.[17]	1/1/1/0/0/1/1/0.5/1/1	**7.5**	1/1/1/0/0/0/1/1/0/0/1	**13.5/21**
2007/ Ma et al.[41]	1/0.5/1/0.5/0/1/1/0.5/1/1	**7.5**	1/1/1/1/0/0.5/1/1/0/0/1	**15/21**
2007/ Rodeo et al.[18]	1/1/1/0/0/1/1/1/1/1	**8**	1/1/1/1/0.5/0.5/1/1/0/0/1	**16/21**
2008/ Sasaki et al.[22]	1/0.5/1/1/1/0.5/1/1/1/1	**9**	1/1/1/1/1/0/1/1/0/0/0.5	**16.5/21**
2008/ Gulotta et al.[19]	1/1/1/0/0/1/1/1/1/1	**8**	1/1/1/1/0.5/1/1/1/0.5/0/1	**17/21**
2009/ Wen et al[32]	1/0.5/1/0/0/1/1/1/1/1	**7.5**	1/1/1/1/0/0/1/0.5/1/0/0.5	**14.5/21**
2009/Mutsuzaki et al.[20]	1/1/1/0/0/1/1/1/1/1	**8**	0.5/1/1/1/0/0/1/1/0/0/1	**14.5/21**
2011/ Pan et al.[33]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/0.5/0/0/1/1/0/0/0	**12.5/21**
2011/Mutsuzaki et al[28]	1/0.5/1/0/0.5/1/1/0.5/1/1	**7.5**	1/1/1/1/0/0/1/1/0/0/1	**14.5/21**
2013/Oka et al.[37]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	0.5/1/1/1/0/0/1/0.5/0/0/0.5	**13/21**
2014/ Kuang et al.[24]	1/0.5/1/0.5/1/1/1/1/1/1	**9**	1/1/1/1/0/0/1/1/0.5/0/0.5	**16/21**
2015/Cheng et al.[25]	1/0.5/1/0.5/1/1/1/1/1/1	**9**	1/1/1/1/0/0/0.5/1/0/0/1	**15.5/21**
2015/Han et al[38]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/0.5/0/0/0.5/0.5/0/0/1	**12.5/21**
2016/Mutsuzaki et al.[26]	1/0.5/1/0.5/1/1/0.5/1/1/1	**8.5**	1/1/1/1/0/0/1/1/0/0/1	**15.5/21**
2018/Lu et al.[23]	1/0.5/1/1/1/1/1/0.5/1/1	**9**	1/1/1/1/0.5/0.5/1/0.5/0.5/0/1	**17/21**
2018/ Wang et al.[39]	1/0.5/1/0/0/0.5/1/0.5/1/1	**6.5**	1/1/1/0.5/0/0/1/1/0.5/0/0.5	**13/21**
2019 / Fu et al.[30]	1/0.5/1/0.5/0/0.5/1/1/1/1	**7.5**	1/1/1/1/0.5/0/1/1/0/0/0.5	**14.5/21**
2019/ Han et al[35]	1/0.5/1/0.5/0/1/1/1/1/1	**8**	1/1/1/1/0/0/0.5/1/0/0/1	**14.5/21**
2019/ Zhang et al.[21]	1/1/1/0.5/1/1/1/0.5/1/1	**9**	1/1/1/0.5/0/0/1/1/0.5/0/1	**16/21**
2020/ Sun et al.[40]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/1/0/0/0.5/1/0/1/1	**14.5/21**
2020/ Wei et al[34]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/1/0.5/0.5/1/0.5/0/0/1	**14.5/21**

Table 2: Characteristics of the animal models of ACLR, materials tested and strategies of tendon graft-bone interface enhancements in the included studies

Author / Year	Animal species	N	Tunnels (Ø x length, mm) *	Graft type (Ø, length, mm)	Graft fixation (Fem + Tib)	Device evaluated	Drug	Strategy	Controls	Follow-up (min-max) (weeks)
2001/ Anderson et al.[16]	Rabbit	70	Ø 1.7	ST: length 38	Periosteum	Collagen sponge	BMP matrix (35 µg)	Wrap-around graft	Sponge alone / Graft alone	2-8

2004/ Tien et al.[42]	Rabbit	22	Θ 2.4	ST	Fem.: LCL (native Tib attachment)	CPC (0.5 ml)	None	Injection	Graft only	1-24
2004/Mihelic et al.[36]	Sheep	50	Θ 4.5	Peroneus tertius	Suspension	Collagen sponge	BMP-7 (25 µg)	Wrap-around graft	Sponge only	3-6
2004/Mutsuzaki et al.[31]	Rabbit	15	Θ 3.2	FDL: Θ 3-4, length 30	Cortical buttons	Ca + P solutions	None	Immersion	Saline solution	3 days – 4 weeks
2005/ Yamazaki et al.[27]	Dog	21	Tib Θ 4, Fem: UN	FDS, Θ 4, length 15. (tib)	Suspension	Fibrin glue (0.1 ml) in tibial tunnel only	TGF-B1 (2 ng)	Injection	Fibrin glue only / Graft only	3
2007 / Huangfu et al.[29]	Dog	48	Fem.: Θ 4.5 Tib: Θ 4.5-5.5	FDL, Θ 4.5, length 40.	Suspension	TCP powder (2.5 g) + sodium phosphate solution (1.4 ml) (2 mL)	None	Injection	Graft	2–12
2007/Walsh et al.[17]	Sheep	82	Θ 8	LDE length 30	Fem.: PLLA I/S (BioRCI®) Tib: PLC or PLLA screw	Resorbable I/S PLC (65% PDLA, 35% $CaCO_3$): Tib. fixation	None	Tib. graft fixation with I/S (PLC)	I/S Tib (PLLA)	6-52
2007/ Ma et al.[41]	Rabbit	60	Θ 2.4	ST	Periosteum	CPM	BMP2 (115 µg)	Injection	CPM + Noggin (30 ng) / CPM alone /	2-8
2007/ Rodeo et al.[18]	Rabbit	60	Θ 2.4	ST	LCL and MCL	CPM (50 µL)	OPG (100 µg / tunnel)	Injection	CPM + RANKL (10 µg / tunnel) CPM Graft only	2-8
2008/ Sasaki et al.[22]	Dog	28	Θ 4	FDS, length 15 (Tib)	Suspension	Gelatin hydrogel sheets 15 x 4 x 0.25 mm	G-CSF (5 µg)	Wrap-around graft	Hydrogel + PBS (20 µL)	2-4
2008/ Gulotta et al.[19]	Rabbit	35	Θ 2.78 x 20	ST	Periosteum & soft tissues	Glue made with Mg (41%) (+Ca and P) (12.5 g)	No	Injection	Graft only	3-6
2009/ Wen et al[32]	Rabbit	28	Θ 2.7	LDE Θ 2	Soft tissues	CPC + Brushite (BCPC)	None	Injection	Graft only	6-12
2009/Mutsuzaki et al.[20]	Goat	20	Θ 6.5 x 20	FDL (Allograft) + Ham., length 45	Fem.: EndoButton® Tib: I/S Θ 4.5 mm	Ca + P solutions	None	Immersion	Saline solution	6
2011/ Pan et al.[33]	Rabbit	51	Θ 2.5	LDE Θ 2, length 30	Periosteum	CPC	BMP matrix	Injection	Fibrin glue + BMP/ Graft only	2-12

2011/Mutsuzaki et al[28]	Goat	12	Θ 6.5 x 20	FDL / Ham., Θ 6.5, length 45	Fem.: EndoButton® Tib, I/S. Θ 4.5 mm	Ca + P solutions	None	Immersion	Saline solution	26
2013/Oka et al.[37]	Rabbit	42	Θ 2.5	ST	Periosteum & soft tissues	Gelatin hydrogel	Simvastatin (125 µg / tunnel)	Wrap-around graft	Hydrogel only	2-8
2014/ Kuang et al.[24]	Rabbit	15	Θ 2 x 10	Achilles (allograft), Θ 2	Sr-CPC + suspension (2.7 mm Θ)	CPC + Strontium (Sr-CPC)	None	Immersion (intra-tunnel portion) + injection	CPC	3-24
2015/Cheng et al.[25]	Rabbit	60	Θ 2.1	ST	Fem.: I/S Tib: periosteum	I/S Mg (99.98% wt.) Θ2.7 x 12 mm length	None	Fem. graft fixation with I/S (Mg)	I/S Ti	3-12
2015/Han et al[38]	Rabbit	24	Θ 2.5	ST: length 30	Periosteum & soft tissues	Mb Nf Es PCL + nano-HA+ Collagen	None	Wrap-around graft	Graft only	4-8
2016/Mutsuzaki et al.[26]	Goat	15	Θ 7 x 15	FDL, Θ 7, length 40	Fem.: EndoButton® Tib: V.I. Θ 4.5 mm	Ca + P solutions	None	Immersion	Saline solution/ Native ACL	26
2018/Lu et al.[23]	Rabbit	84	Θ 1.2	LDE	Suspension (fem) suture (tib)	Collagen solution (15 mL)	FGF1: 4 µg or 1 µg	Injection (+thrombin)	Collagen / Graft only	4-12
2018/ Wang et al.[39]	Rabbit	48	Θ 2.5	LDE, length 30	Fem.: I/S Tib.: soft tissue	I/S MgZnSr Θ 3 x 8 mm length	No	Fem. fixation: I/S	I/S (PLA)	0-16
2019 / Fu et al.[30]	Dog	21	Θ 4	FDL, Θ 4, length 40	Implant in Fem + Tib. tunnels	Alloy ZK60 Mg Bio-Transfix (12 x 2 mm) porous, resorbable	BMP2	Fixation with implant	Implant w/o BMP2/ Non-porous implant	4 days – 12 weeks
2019/ Han et al[35]	Rabbit	48	Θ 2.5	ST	Periosteum & soft tissues	Mb Nf Es PCL + multilayer + chitosan + Ac. Hyaluronic	SDF-1 + BMP2	Wrap-around graft	Mb PCL/ Mb PCL + BMP2	4-8
2019/ Zhang et al.[21]	Rabbit	18	Θ 2.5	ST	T/O suture (bone bridge) + soft tissue	Gelatin sponge 5 x 5 x 2 mm	PRP (1 mL)	Wrap-around graft	Sponge/ PRP	8
2020/ Sun et al.[40]	Rabbit	60	Θ 2.5	LDE, length 30	Fem.: I/S studied Tib.: T/O suture	I/S alloy ZnMnMg (Θ 1.9-3 x 8 mm length)	None	Fem. graft fixation with I/S	I/S Ti	6-16
2020/ Wei et al[34]	Rabbit	60	Θ 2 (w/o sponge) 2.5 Θ (w/ sponge)	Achilles Θ 1.3–1.6	T/O suture	Collagen sponge (10 x 5 mm)	Solution OPG (1 mg) + BMP2 (1 µg): (100 µg/mL)	Wrap-around graft	Graft only / sponge / OPG + BMP2	4-12

Fem: femoral; Tib: tibial; UN: unknown; LDE: long digital extensor; ST: Semitendinosus, FDL: flexor digitorum longus; FDS: flexor digitorum superficialis; LCL: lateral collateral ligament; MCL: medial collateral ligament; I/S: interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; PGA: propylene glycol alginate; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor; SDF 1: stromal cell-derived factor 1; PBS: phosphate buffered saline; ctrl: control group; T/O: transosseous * femoral and tibial tunnel identical unless specified

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14 Table 3: Main quantitative histological results of the included studies

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Year / Author Animal/ Strategy	Study design	Histological analysis methods	Results vs. controls at each time-point*				
			Before 2 weeks	2-3 weeks	4-6 weeks	8 weeks	12 weeks and after
2004/Mihelic et al.[36] Sheep/ Wrap-around	Collagen + BMP7 vs Collagen	Histomorphometry: Bone volume (B.V., %) trabecular thickness (T.T., µm), number (T.N. per mm), Separation (T.S., µm), Tendon fiber outgrowth (T.F.O., µm)		**B.V.:** **Collagen+BMP7: 41,5** **Collagen: 30,6** **(p< 0,05)** T.T.: N.S. (119,5-131,6) **T.N.:** **Collagen+BMP7: 3,2** **Collagen: 2,6** **(p< 0,05)** **T.S.:** **Collagen+BMP7: 189** **Collagen:** **277,4** **(p< 0,05)** **T.F.O.: Collagen+BMP7:** **995** **Collagen: 486** **(p< 0,01)**			
2008/ Sasaki et al.[22] Dog/ Wrap-around	Gelatin + G-CSF vs Gelatin	Quantitative Immunohistology (number of capillaries / fields of view)		**Gelatin+G-CSF: 781,5** **Gelatin:** **316,5 (p <0,01)**			
2013/Oka et al.[37] Rabbit/ Wrap-around	Gelatin + Simvastatin vs Gelatin	Quantitative Immunohistology (number of capillaries and osteoblasts / mm²)		***Capillaries*** **Gelatin + Simvastatin: 112** **Gelatin: 72 (p<0,01)** ***Osteoblasts*:** **Gelatin+Simvastatin: 495** **Gelatin: 272 (p<0,001)**			

2019/ Zhang et al.[21] Rabbit/ Wrap-around	Gelatin+PRP vs PRP vs Gelatin	Semi-quantitative score (Tan et al. / 10)			**Gelatin+PRP: 7,83** **PRP: 6,17 (p = 0,039)** **Gelatin: 5,17 (p=0,003)**	
2020/ Wei et al[34] Rabbit/ Wrap-around	Collagen +OPG + BMP2 vs Collagen vs OPG + BMP2 vs Graft	Histomorphometry: Tunnels Enlargement (mm) New bone area (mm²)		*Tunnels enlargement*: N.S. vs graft *NB area:* N.S. vs graft	***Tunnels enlargement:*** **Collagen + OPG+BMP2: 0,45 (fem), 0,42 (tib)** **Graft: 0,70 (fem) (p<0,01), 0,80 (tib) (p<0,01)** ***NB area:*** **Collagen + OPG+BMP2: 0,38** **Graft: 0,21 (p<0,01)**	***Tunnels enlargement:*** **Collagen + OPG+BMP2 : 0,44 (fem), 0,41 (tib)** **Graft: 0,80 (fem (p<0,01), 0,87 (tib) (p< 0,01)** ***NB area:*** **Collagen + OPG+BMP2: 0,45** **Graft: 0,32 (p < 0,01)**
		Yamakado et al. score (four items scored from 0 to 3)		Better in collagen + OPG + BMP2 group (no stats)	Better in Collagen + OPG+BMP2 group (no stats)	Better in Collagen + OPG+BMP2 group (no stats)
2005/ Yamazaki et al.[27] Dogs/ Injection	Fibrin (tib.) + TGF-B1 vs fibrin vs Graft	Quantitative: Bone ingrowth in tunnel (%)	Fibrin + TGF-B1: 55-65% Graft: 30-40% (no stats)			
2007/ Ma et al.[41] Rabbit/ Injection	CPM + BMP2 vs CPM + Noggin vs CPM	Histomorphometry: New bone ingrowth (mm)	**CPM+BMP2 :0,30 81% > control (CPM) (p<0,05)**	**CPM+BMP2: 0,32: 89% > control (CPM) (p<0,05)**	**CPM+BMP2 :0,31 113% > control (CPM) (p<0,05)**	
2007/ Rodeo et al.[18] Rabbit/ Injection	CPM + OPG vs CPM + RANKL vs CPM vs Graft	Histomorphometry: New bone ingrowth (mm)	**CPM + OPG: 0,19 (p=0,004)** **Control (CPM): 0,1**	N.S. (0,12-0,19)	**CPM + OPG: 0,2 (p= 0,033)** **Control (CPM): 0,11**	
		Immunohistology (number of osteoclasts / mm of tunnel)	**CPM+OPG: 1 / mm (p = 0,014)** **Control (CPM): 5 / mm**	**CPM+OPG: 4 / mm (p = 0,02)** **Control (CPM): 8/ mm**	**CPM+OPG: 4 / mm (p > 0,05)** **Control (CPM): 4/ mm**	

2008/ Gulotta et al.[19] Rabbit/ Injection	Mg + Ca + P Glue vs Graft	Histomorphometry: Interface width (µm)	N.S. (145-190 fem, 145-149 tib)	***Fem.:*** **Mg+Ca+P: 70 (p=0,04)** **Graft: 157** ***Tib.:*** **Mg+Ca+P: 76 (p=0,04)** **Graft: 150**		
2009/ Wen et al[32] Rabbit/ Injection	BCPC vs Graft	Quantitative: Fluorescence: new bone formation (µm/week)		N.S. (17-19)		N.S. (no data)
2011/ Pan et al.[33] Rabbit/ Injection	CPC + BMP vs Fibrin + BMP vs Graft	Quantitative Fluorescence: bone mineralization rate (µm / day)		**CPC+BMP: 2,9** **Graft: 2,3** **(p<0,05)**		**CPC+BMP: 3** **Graft: 2,1** **(p<0,05)**
2014/ Kuang et al.[24] Rabbit/ Injection	Sr-CPC vs CPC	Score Yeh et al. (new bone, FC, graft connection, each from 0 to 3, total from 0 to 9)	**CPC: 1,2** **Sr-CPC: 1,9 (p<0,001)**	**CPC: 2** **Sr-CPC: 3,3 (p<0,001)**	**CPC: 2,7** **Sr-CPC: 4,6 (p<0,001)**	**12 weeks:** **Sr-CPC: 6,6 (p<0,001)** **CPC: 4,1**
2007/Walsh et al.[17] Sheep / Tib. Graft fixation	PLC I/S vs PLLA I/S	Semi-quantitative score: new bone ingrowth (0 to 4)		N.S.		24 weeks: N.S. (6,7-6,8) **12 weeks:** **PLC: 1,5/4** **PLLA = 0/4 (p<0,05)** **26 weeks:** **PLC: 3,5/4** **PLLA: 0/4 (p<0,05)** **52 weeks:** **PLC: 4/4 (p<0,05)** **PLLA: 0/4**
2015/Cheng et al.[25] Rabbit/ Fem. Graft Fixation	Mg I/S vs Ti I/S	Semi quantitative (FC interface, %)	N.S.	N.S.	**Mg :35% (p<0,05)** **Ti: 22%**	**Mg: 60% (p<0,01)** **Ti: 40%**
		Immunohistology (BMP2 and VEGF detection)		**BMP2: Mg > Ti (p<0,05)**		N.S.
		Score Kuang et al. (3 items from 0 to 3, total: 0 to 9)	N.S.	N. S	**Mg: 5,1 (p<0,05)** **Ti: 3,7**	**Mg: score 7/9 (p<0,05)** **Control (Ti): 5,3**
2011/Mutsuzaki et al[28] Sheep/ Immersion	Ca + P solutions vs Graft	Quantitative Cartilage formation (%)				At 26 weeks **CaP: 37,5% (p=0,0416)** **Graft: 8%**

		Number of osteoclasts / mm	***Fem.:*** **CaP: 0,29** **Graft: 1,68 (p<0,05)** *Tib.:* N.S. (0,29-0,43)
2016/Mutsuzaki et al.[26] Sheep/ Immersion	Ca + P solutions vs Graft	Histomorphometry: Cartilage area (mm^2) Nonbonding gap in BTI (mm)	At 26 weeks Cartilage area: CaP: 0,06 to 0,17 **(p=0,009, fem)** (N.S., tib) Graft: 0,02 to 0,28 Nonbonding gap: CaP: 0,6 to 1,1 Control: 1,5 to 3,1 (p=0,11, fem) **(p=0,047, tib.)**
		score Murray et al. (cells, extracellular matrix and vascular characteristics, total /28)	N.S. (20,8-22,8)

Fem.: femoral; Tib: tibial; FC : fibrocartilage; NB : new bone; SF : Sharpey fibers ; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor; SDF 1: stromal cell-derived factor 1; VEGF: Vascular Endothelial Growth Factor ; N.S.: not significant; BTI: bone-tendon interface;* Mean results. Significative results were written in bold.

Table 4: Main biomechanical results of the included studies

Year / Author Animal/ Strategy	Study design Strain rate	Biomechanical analysis methods	Results at each time-point*				
			Before 2 weeks	2-3 weeks	4-6 weeks	8 weeks	12 weeks and after
2001/ Anderson et al.[16] Rabbit/Wrap-around	Collagen +BMP vs Collagen vs Graft 40 mm/sec	UFL (N)		**Collagen + BMP: 54,7** **Graft: 37,3** **(p = 0,04)**	**Collagen + BMP: 65,8** **Graft: 39,4** **(p = 0,01)**	**Collagen + BMP: 70,7** **Graft: 39,4** **(p < 0,001)**	

		Tunnel failure (%)	Collagen + BMP: 85% Graft: 92% N.S.	Collagen + BMP: 69% Graft: 54% N.S.	Collagen + BMP: 55% Graft: 31% N.S.
2004/Mihelic et al.[36] Sheep /Wrap-around	Collagen + BMP7 vs Collagen 0,1N / sec	UFL (N)	**Collagen + BMP: 350** **Collagen: 212** **(p< 0,01)**	**Collagen + BMP: 380** **Collagen: 215** **(p< 0,01)**	
2008/ Sasaki et al.[22] Dog/Wrap-around	Gelatin + G-CSF vs Gelatin 20mm / min	UFL (N)	N.S. (25,5-27,2)	**Gelatin + G-CSF: 99,5** **Gelatin: 32** **(p< 0,01)**	
		Stiffness (N/mm)	Gelatin+G-CSF 19,6 Gelatin: 15,2 (no stats)	Gelatin+G-CSF 25,5 Gelatin 11,9 (no stats)	
		Tunnel failure (%)	Both groups: 100 %	Gelatin+G-CSF: 16,7% Gelatin: 100%	
2013/Oka et al.[37] Rabbit/Wrap-around	Gelatin + Simvastatin vs Gelatin 10mm / min	UFL (N)	**Gelatin+ Simvastatin: 32,5** **Gelatin: 21,6** **(p<0,05)**	N.S. (33,4-33,5)	N.S. (38,4-36,7)
		Stiffness (N/mm)	N.S. (8,9-12,8)	N.S. (11,1-13,6)	N.S. (15,3-16,3)
		Tunnel failure (%)	Both groups: 100%	Both groups: 33%	Both groups: 33%
2015/Han et al[38] Rabbit/Wrap-around	Mb PCL / nano-HA/ Collagen vs Graft 2 mm / min	UFL (N)		N.S. (26-28)	**Mb PCL: 58,4** **Graft: 39,9** **(p<0,001)**
		Stiffness (N/mm)		N.S. (7-8)	**Mb PCL: 15,2** **Graft: 10,2** **(p<0,001)**
		Tunnel failure (%)		Both groups: 100%	Both groups: 100%
2019/ Han et al[35]	Mb multilayer + SDF1 + BMP2	UFL (N)		N.S. (16-31)	**Mb multilayer + SDF1+BMP2: 79,9**

Rabbit/Wrap-around	vs Mb PCL + BMP2 vs Mb PCL 5 mm / min	Stiffness (N/mm)			N.S. (6-7)	**Mb PCL: 63,5 (p<0,05)** **Mb multilayer + SDF1+BMP2: 19,5** **Mb PCL: 10,8 (p<0,05)**
2019/ Zhang et al.[21] Rabbit/Wrap-around	Gelatin+PRP vs PRP vs Gelatin 20 mm / min	UFL (N) Stiffness (N/mm) Tunnel failure (%)				**Gelatin + PRP: 42,7** **Gelatin: 36,9 (p=0,041)** **Gelatin + PRP: 3,2** **Gelatin: 2 (p=0,017)** **All groups: 100%**
2004/ Tien et al.[42] Rabbit/Injection	CPC vs Graft 5 mm /second	UFL (N) Tunnel failure (%)	**At 1 week** **CPC: 6,5** **Graft: 2 (p=0,027)** Both groups: 100%	**CPC: 11,5** **Graft: 5,4 (p=0,028)** Both groups: 100%		
2005/ Yamazaki et al.[27] Dogs/Injection	Fibrin (tib.) + TGF-B1 vs fibrin vs Graft 20 mm /min	UFL (N) Stiffness (N/mm) Tunnel failure (%)		**Fibrin+TGF-B1: 188,2** **Graft: 87,4 (p=0,003)** **Fibrin+TGF-B1: 72** **Graft: 33 (p=0,002)** All groups: 100%		
2007 / Huangfu et al.[29] Dogs /Injection	TCP vs Graft Strain 10 mm /min	UFL (N)		**TCP: 29,1** **Graft: 14,4 (p<0,001)**	**4 weeks** **TCP: 62,9** **Graft: 33,6 (p<0,001)** 6 weeks: N.S. (74,8-47,1)	No measured (midsubstance failures)

		Tunnel failure (%)	Both groups: 100%	TCP: 60% Graft: 80%	TCP: 40% Graft: 60%	TCP: 0% Graft: 20%
2007/ Ma et al.[41] Rabbit/Injection	CPM + BMP2 vs CPM + Noggin vs <u>CPM</u> 10 mm /min	UFL (N)	N.S. (20-22)	N.S. (30-38)	N.S. (32-50)	
		Stiffness (N/mm)	N.S. (8-9)	N.S. (12-13)	**CPM + BMP2: 25** **CPM: 11** **(p< 0,05)**	
2007/ Rodeo et al.[18] Rabbit/Injection	CPM + OPG vs CPM + RANKL vs CPM vs <u>Graft</u> 10 mm /min	UFL (N)	N.S. (20-25)	N.S. (38)	N.S. (38-50)	
		Stiffness (N/mm)	N.S. (8-10)	N.S. (11-14)	**CPM+ OPG: 22** **CPM: 10** **(p= 0,017)**	
		Tunnel failure (%)	All groups: 100%	Not described	CPM + OPG: 0% CPM: 100%	
2008/ Gulotta et al.[19] Rabbit/Injection	Mg + Ca + P Glue vs <u>Graft</u> 10 mm/min	UFL (N)	N.S. (36-37)	**Glue: 72** **Graft: 43** **(p=0,04)**		
		Tunnel failure (%)	Both groups: 100%	Both groups: 100%		
2009/ Wen et al[32] Rabbit/Injection	BCPC vs <u>Graft</u> 50 mm/min	UFL (N)		**BCPC: 94** **Graft: 43** **(p<0,05)**		**BCPC: 60** **Graft: 39** **(p<0,05)**
		Stiffness (N/mm)		**BCPC: 31** **Graft: 15** **(p<0,05)**		**BCPC: 22** **Graft: 16** **(p<0,05)**
		Tunnel failure (%)		**BCPC: 75%** **Graft: 100%** **(p=0,035)**		**BCPC: 37,5%** **Graft: 100%** **(p<0,013)**
2011/ Pan et al.[33] Rabbit/Injection	CPC + BMP vs Fibrin + BMP vs <u>Graft</u> 50 mm/min	UFL (N)		**CPC+BMP: 79** **Graft: 43** **(p<0,01)**		N.S. (38-53)
		Tunnel failure (%)		CPC+BMP: 87,5% Graft: 100%		CPC+BMP: 37,5% Graft: 12,5%
2018/Lu et al.[23] Rabbit/Injection		UFL (N)		**Collagen+FGF: 25** **Graft: 17**	N.S. (22-45)	**Collagen+FGF: 75** **Graft: 32**

	Collagen + FGF vs Collagen vs Graft 5 mm /min	Stiffness (N/mm)		**(p<0,05)** **Collagen+FGF: 10** **Graft: 5** **(p<0,05)**	**Collagen+FGF: 7,5** **Graft: 5** **(p<0,05)**	**(p<0,05)** **Collagen+FGF: 5** **Graft: 4** **(p<0,05)**
2007/Walsh et al.[17] Sheep/Tib. Graft Fixation	PLC I/S vs PLLA I/S 50 mm /min	UFL (N) Tunnel failure (%)		N.S. (50-60) Both groups: 0%	N.S. (210-220) Both groups: 0%	
2015/Cheng et al.[25] Rabbit/ Fem. Graft Fixation	Mg I/S vs Ti I/S 0,5 mm /min	UFL (N) Stiffness (N/mm) Tunnel failure (%)	Post-op N.S. (110-115) N.S. (25-27) Both groups: 100%			N.S. (120-130) N.S. (45-55) Both groups: 0%
2018/ Wang et al.[39] Rabbit/ Fem. Graft Fixation	MgZnSr I/S vs PLA I/S 50 mm /min	UFL (N)		N.S. (38-40)		**MgZnSr: 68** **PLA: 38** **(p<0,05)**
2019 / Fu et al.[30] Dogs/Fixation	Porous Mg Bio-Transfix + BMP2 vs porous Mg BioTransfix vs non-porous Mg BioTransfix 1 mm /min	UFL (N)				**MgBioTransfix + BMP2: 251** **MgBioTransfix: 177** **Non-porous: 64** **(p<0,05)**
2020/ Sun et al.[40] Rabbit/ Fem. Graft Fixation	ZnMnMg I/S vs Ti I/S 50 mm /min	UFL (N)		N.S. (50-75)		**12 weeks** **ZnMnMg: 110** **Ti: 90** **(p<0,05)**

				16 weeks: N.S. (115)
		Tunnel failure (%)		12 weeks ZnMnMg: :0% Ti: 50%
2009/Mutsuzaki et al.[20] Sheep/ Immersion	Ca + P solutions vs Graft 30 mm /sec	UFL (N) Stiffness (N/mm) Tunnel failure (%)	N.S. (109-117) N.S. (28-32) CaP: 29% Graft: 43%	
2011/Mutsuzaki et al[28] Sheep/ Immersion	Ca + P solutions vs Graft 30 mm /min	UFL (N) Stiffness (N/mm) Tunnel failure (%)		At 26 weeks N.S. (562-575) N.S. (43,5-50,5) Both groups: 0%
2016/Mutsuzaki et al.[26] Sheep /Immersion	Ca + P solutions vs Graft	Anterior tibial translation at 50N load (mm) Internal rotation (degree) at 2N/m torque at 0°,60°, 90° knee flexion		At 26 weeks Anterior tib. Translation N.S. Internal tibial torque: N.S.

Fem: femoral; Tib: tibial; FC : fibrocartilage; NB : new bone; SF : Sharpey fibers ; UFL : Ultimate Failure Load; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor; SDF 1: stromal cell-derived factor 1; VEGF: Vascular Endothelial Growth Factor ; N.S.: not significant;* Mean results. Significant results are written in bold. When not significant, results were reported as minimal and maximal values between groups, in brackets.

Table 5: Main radiological results of the included studies

Year / Author Animal/ Strategy	Study design	Radiological analysis	Results vs. controls at each time-point*			
			2-3 weeks	4-6 weeks	8 weeks	12 weeks and after

2001/ Anderson et al.[16] Rabbit /Wrap-around	Collagen +BMP vs Collagen vs Graft	Qualitative MRI: NB	NB in 21 tunnels (no comparison)			
2008/ Sasaki et al.[22] Dog/Wrap-around	Gelatin + G-CSF vs Gelatin	CT: bone tunnel area (mm²)	N.S. (25,6-29,9)	**Gelatin+G-CSF: 21,51** **Gelatin: 41,8** **(p< 0,05)**		
2013/Oka et al.[37] Rabbit/Wrap-around	Gelatin + Simvastatin vs Gelatin	Micro-CT: bone tunnel area (mm²)	**Gelatin+Simvastatin: 3,25** **Gelatin: 4,13** **(p<0,05)**	**Gelatin+Simvastatin: 1,61** **Gelatin: 2,71** **(p<0,01)**	**Gelatin+Simvastatin: 0,94** **Gelatin: 1,61** **(p=0,06)**	
2019/ Zhang et al.[21] Rabbit/Wrap-around	Gelatin+PRP vs PRP vs Gelatin	Qualitative MRI			Gelatin: No NB, no FC Gelatin+PRP: presence of FC	
2007/ Ma et al.[41] Rabbit/Injection	CPM + BMP2 vs CPM + Noggin vs CPM	Radiographs : tunnel diameter (mm)	**CPM + BMP2: 1,9** **15% smaller than CPM (p<0,05)**	**CPM + BMP2: 1,6** **25% smaller than CPM (p<0,05)**	**CPM + BMP2: 1,3** **42% smaller than CPM (p<0,05)**	
2007/ Rodeo et al.[18] Rabbit/Injection	CPM + OPG vs CPM + RANKL vs CPM vs Graft	Radiographs: Bone tunnel area (mm²)	N.S. (3,2-4,2)	N.S. (3,4-4,3)	N.S. (2,2-3,6)	
2008/ Gulotta et al.[19] Rabbit/Injection	Mg + Ca + P Glue vs Graft	Micro-CT: Total Bone Volume (mm3) Bone/Tissue Ratio Bone Mineral Tissue Mineral Trabecular Thickness (µm)	All measures N.S. (no data)	Bone Volume: Glue: 27 Graft: 12 (p=0,003, fem.) (N.S. tib.) All other measures N.S. (no data)		
2009/ Wen et al[32] Rabbit/Injection	BCPC vs Graft	Micro-CT: Bone/Tissue Volume Ratio (BV/TV) Trabecular Thickness (TT, mm)		BV/TV: Tib.: **BCPC: 0,084** **Graft:0,045** **(p<0,05)** Fem: N.S. TT: N.S.		BV/TV: **Tib. /Fem.** **BCPC: 0,087 / 0,144** **Graft:0,060 / 0,064** **(p<0,05)** TT: N.S.

2011/ Pan et al.[33] Rabbit/Injection	CPC + BMP vs Fibrin + BMP vs Graft	Micro-CT: Bone mineral density (mg/cm3)	**CPC+BMP: 93** **Graft: 69** **(p<0,05)**			N.S. (109-125)
2007/Walsh et al.[17] Sheep /Tib. Graft fixation	PLC I/S vs PLLA I/S	Qualitative CT		PLC: NB, yes PLLA: NB, no		At 12 weeks PLC: NB, yes PLLA: NB, no At 26 and 52 weeks PLC screw undetectable, replaced by NB PLLA screw intact, limited NB
2015/Cheng et al.[25] Rabbit /Fem. Graft fixation	Mg I/S vs Ti I/S	Qualitative micro-CT				Mg screw: corrosion + mineral deposition Ti screw: intact
2018/ Wang et al.[39] Rabbit /Fem. Graft fixation	MgZnSr I/S vs PLA I/S	Micro-CT Bone volume (BV, mm3) Trabecular thickness (Tb.Th., mm), trabecular number, trabecular separation	**BV:** **MgZnSr: 3,3** **PLA: 1,75** **(p<0,05)** **Tb. Th:** **MgZnSr: 0,27** **PLA: 0,17** **(p<0,05)**			**At 12 weeks** **BV:** **MgZnSr: 3,2** **PLA: 1,4** **(p<0,05)** **Tb.Th.:** **MgZnSr: 0,32** **PLA: 0,17** **(p<0,05)** At 16 weeks: MgZnSr: Replaced by NB **BV:** **MgZnSr: 2,9** **PLA: 1,2** **(p<0,05)** **Tb.Th.:** **MgZnSr :0,26** **PLA: 0,16** **(p<0,05)**
2019 / Fu et al.[30] Dogs/Fixation with implant	Porous Mg Bio-Transfix + BMP2 vs porous Mg BioTransfix vs	Qualitative X-Ray, MRI, Micro-CT		MgBioTransfix + BMP2: NB, yes	MgBioTransfix + BMP2: NB, yes, > controls	MgBioTransfix+BMP2: replaced by NB, NB formation: N.S.

	non-porous Mg BioTransfix			
2020/ Sun et al.[40] Rabbit/Fem. graft fixation with I/S	ZnMnMg I/S vs Ti I/S	Micro-CT: Bone volume/Total volume ratio (BV/TV) Trabecular number (TbN / mm)	**BV/TV: ZnMnMg: 0,6 Ti: 0,5 (p<0,05)** **TbN: ZnMnMg: 3,1 Ti: 2,9 (p<0,05)**	At 12 weeks: N.S. At 16 weeks: N.S.
2011/Mutsuzaki et al[28] Sheep/Immersion	Ca + P solutions vs Graft	Micro-CT: Tunnel diameter (mm) Tunnel area (mm²) Tunnel enlargement (%)		At 26 weeks: Tunnel enlargement: Fem. CaP: 118% Graft: 170% (p=0,027) Tib. N.S. (66-114%)
2016/Mutsuzaki et al.[26] Sheep/Immersion	Ca + P solutions vs Graft	Micro-CT Tunnel area (mm²) Tunnel enlargement (%)		At 26 weeks Tunnel enlargement: N.S. for fem. (28-51%) and tib. (-2,8 – 2,9%)

Fem.: femoral; Tib: tibial; FC: fibrocartilage; NB: new bone; SF: Sharpey fibers; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; CPM: Calcium phosphate matrix; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; 1; N.S.: not significant; * Mean results. Significant results are written in bold. When not significant, results were reported as minimal and maximal values between groups, in brackets.

Year/Authors	ARRIVE Essential 10 (1 = total, 0.5 = partial, 0 = none)	Essential 10 Score (/10)	ARRIVE Recommended Set (11 items) (1 = total, 0.5 = partial, 0 = none)	Total Score (/21)
2001/ Anderson et al.[16]	1/1/1/0.5/0/1/1/0/1/1	**7.5**	1/1/1/1/0.5/0/1/1/0/0/0.5	**14.5/21**
2004/ Tien et al.[42]	1/0.5/1/0/0/1/1/1/1/1	**7.5**	1/1/1/0/0.5/0/0.5/1/0/0/1	**13.5/21**
2004/Mihelic et al.[36]	1/0.5/1/0.5/0/1/0.5/1/1/1	**7.5**	1/1/1/0.5/0/0/0.5/1/0/0/1	**13.5/21**
2004/Mutsuzaki et al.[31]	1/0.5/1/0/0/1/0.5/0.5/1/1	**7**	1/1/1/1/0/0/0.5/0.5/0/0/0.5	**12.5/21**
2005/ Yamazaki et al.[27]	1/0.5/1/0/0.5/1/1/0.5/1/1/	**7.5**	1/1/1/0.5/0.5/0/1/1/0.5/0/0.5	**14.5/21**
2007 / Huangfu et al.[29]	1/0.5/1/0.5/0/0.5/0/1/1/1	**6.5**	1/1/1/1/1/0/1/1/0/0/0.5	**14/21**
2007/Walsh et al.[17]	1/1/1/0/0/1/1/0.5/1/1	**7.5**	1/1/1/0/0/0/1/1/0/0/1	**13.5/21**
2007/ Ma et al.[41]	1/0.5/1/0.5/0/1/1/0.5/1/1	**7.5**	1/1/1/1/0/0.5/1/1/0/0/1	**15/21**
2007/ Rodeo et al.[18]	1/1/1/0/0/1/1/1/1/1	**8**	1/1/1/1/0.5/0.5/1/1/0/0/1	**16/21**
2008/ Sasaki et al.[22]	1/0.5/1/1/1/0.5/1/1/1/1	**9**	1/1/1/1/1/0/1/1/0/0/0.5	**16.5/21**
2008/ Gulotta et al.[19]	1/1/1/0/0/1/1/1/1/1	**8**	1/1/1/1/0.5/1/1/1/0.5/0/1	**17/21**
2009/ Wen et al[32]	1/0.5/1/0/0/1/1/1/1/1	**7.5**	1/1/1/1/0/0/1/0.5/1/0/0.5	**14.5/21**
2009/Mutsuzaki et al.[20]	1/1/1/0/0/1/1/1/1/1	**8**	0.5/1/1/1/0/0/1/1/0/0/1	**14.5/21**
2011/ Pan et al.[33]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/0.5/0/0/1/1/0/0/0	**12.5/21**
2011/Mutsuzaki et al[28]	1/0.5/1/0/0.5/1/1/0.5/1/1	**7.5**	1/1/1/1/0/0/1/1/0/0/1	**14.5/21**
2013/Oka et al.[37]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	0.5/1/1/1/0/0/1/0.5/0/0/0.5	**13/21**
2014/ Kuang et al.[24]	1/0.5/1/0.5/1/1/1/1/1/1	**9**	1/1/1/1/0/0/1/1/0.5/0/0.5	**16/21**
2015/Cheng et al.[25]	1/0.5/1/0.5/1/1/1/1/1/1	**9**	1/1/1/1/0/0/0.5/1/0/0/1	**15.5/21**
2015/Han et al[38]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/0.5/0/0/0.5/0.5/0/0/1	**12.5/21**
2016/Mutsuzaki et al.[26]	1/0.5/1/0.5/1/1/0.5/1/1/1	**8.5**	1/1/1/1/0/0/1/1/0/0/1	**15.5/21**
2018/Lu et al.[23]	1/0.5/1/1/1/1/1/0.5/1/1	**9**	1/1/1/1/0.5/0.5/1/0.5/0.5/0/1	**17/21**
2018/ Wang et al.[39]	1/0.5/1/0/0/0.5/1/0.5/1/1	**6.5**	1/1/1/0.5/0/0/1/1/0.5/0/0.5	**13/21**
2019 / Fu et al.[30]	1/0.5/1/0.5/0/0.5/1/1/1/1	**7.5**	1/1/1/1/0.5/0/1/1/0/0/0.5	**14.5/21**
2019/ Han et al[35]	1/0.5/1/0.5/0/1/1/1/1/1	**8**	1/1/1/1/0/0/0.5/1/0/0/1	**14.5/21**
2019/ Zhang et al.[21]	1/1/1/0.5/1/1/1/0.5/1/1	**9**	1/1/1/0.5/0/0/1/1/0.5/0/1	**16/21**
2020/ Sun et al.[40]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/1/0/0/0.5/1/0/1/1	**14.5/21**
2020/ Wei et al[34]	1/0.5/1/0/0/1/1/0.5/1/1	**7**	1/1/1/1/0.5/0.5/1/0.5/0/0/1	**14.5/21**

Table 1: Evaluation of the methodological quality of the included studies

Table 2: Characteristics of the animal models of ACLR, materials tested and strategies of tendon graft-bone interface enhancements in the included studies

Author / Year	Animal species	N	Tunnels (Θ x length, mm) *	Graft type (Θ, length, mm)	Graft fixation (Fem + Tib)	Device evaluated	Drug	Strategy	Control
2001/ Anderson et al.[16]	Rabbit	70	Θ 1.7	ST: length 38	Periosteum	Collagen sponge	BMP matrix (35 µg)	Wrap-around graft	Sponge / Graft
2004/ Tien et al.[42]	Rabbit	22	Θ 2.4	ST	Fem.: LCL (native Tib attachment)	CPC (0.5 ml)	None	Injection	Graft o
2004/Mihelic et al.[36]	Sheep	50	Θ 4.5	Peroneus tertius	Suspension	Collagen sponge	BMP-7 (25 µg)	Wrap-around graft	Sponge
2004/Mutsuzaki et al.[31]	Rabbit	15	Θ 3.2	FDL: Θ 3-4, length 30	Cortical buttons	Ca + P solutions	None	Immersion	Saline solution
2005/ Yamazaki et al.[27]	Dog	21	Tib Θ 4, Fem: UN	FDS, Θ 4, length 15. (tib)	Suspension	Fibrin glue (0.1 ml) in tibial tunnel only	TGF-B1 (2 ng)	Injection	Fibrin g only / C only
2007 / Huangfu et al.[29]	Dog	48	Fem.: Θ 4.5 Tib: Θ 4.5-5.5	FDL, Θ 4.5, length 40.	Suspension	TCP powder (2.5 g) + sodium phosphate solution (1.4 ml) (2 mL)	None	Injection	Graft
2007/Walsh et al.[17]	Sheep	82	Θ 8	LDE length 30	Fem.: PLLA I/S (BioRCI®) Tib: PLC or PLLA screw	Resorbable I/S PLC (65% PDLA, 35% $CaCO_3$): Tib. fixation	None	Tib. graft fixation with I/S (PLC)	I/S Tib (PLLA
2007/ Ma et al.[41]	Rabbit	60	Θ 2.4	ST	Periosteum	CPM	BMP2 (115 µg)	Injection	CPM + Noggin

									(30 ng) CPM a
2007/ Rodeo et al.[18]	Rabbit	60	Θ 2.4	ST	LCL and MCL	CPM (50 µL)	OPG (100 µg / tunnel)	Injection	CPM + RANK (10 µg tunnel) CPM Graft o
2008/ Sasaki et al.[22]	Dog	28	Θ 4	FDS, length 15 (Tib)	Suspension	Gelatin hydrogel sheets 15 x 4 x 0.25 mm	G-CSF (5 µg)	Wrap-around graft	Hydrog PBS (2
2008/ Gulotta et al.[19]	Rabbit	35	Θ 2.78 x 20	ST	Periosteum & soft tissues	Glue made with Mg (41%) (+Ca and P) (12.5 g)	No	Injection	Graft o
2009/ Wen et al[32]	Rabbit	28	Θ 2.7	LDE Θ 2	Soft tissues	CPC + Brushite (BCPC)	None	Injection	Graft o
2009/Mutsuzaki et al.[20]	Goat	20	Θ 6.5 x 20	FDL (Allograft) + Ham., length 45	Fem.: EndoButton® Tib: I/S Θ 4.5 mm	Ca + P solutions	None	Immersion	Saline solutio
2011/ Pan et al.[33]	Rabbit	51	Θ 2.5	LDE Θ 2, length 30	Periosteum	CPC	BMP matrix	Injection	Fibrin g BMP/ Graft o
2011/Mutsuzaki et al[28]	Goat	12	Θ 6.5 x 20	FDL / Ham., Θ 6.5, length 45	Fem.: EndoButton® Tib, I/S. Θ 4.5 mm	Ca + P solutions	None	Immersion	Saline solutio
2013/Oka et al.[37]	Rabbit	42	Θ 2.5	ST	Periosteum & soft tissues	Gelatin hydrogel	Simvastatin (125 µg / tunnel)	Wrap-around graft	Hydrog only
2014/ Kuang et al.[24]	Rabbit	15	Θ 2 x 10	Achilles (allograft), Θ 2	Sr-CPC + suspension (2.7 mm Θ)	CPC + Strontium (Sr-CPC)	None	Immersion (intra-tunnel portion) + injection	CPC
2015/Cheng et al.[25]	Rabbit	60	Θ 2.1	ST	Fem.: I/S Tib: periosteum	I/S Mg (99.98% wt.) Θ2.7 x 12 mm length	None	Fem. graft fixation with I/S (Mg)	I/S Ti
2015/Han et al[38]	Rabbit	24	Θ 2.5	ST: length 30	Periosteum & soft tissues	Mb Nf Es PCL + nano-HA+ Collagen	None	Wrap-around graft	Graft o
2016/Mutsuzaki et al.[26]	Goat	15	Θ 7 x 15	FDL, Θ 7, length 40	Fem.: EndoButton® Tib: V.I. Θ 4.5 mm	Ca + P solutions	None	Immersion	Saline solutio Native
2018/Lu et al.[23]	Rabbit	84	Θ 1.2	LDE	Suspension (fem) suture (tib)	Collagen solution (15 mL)	FGF1: 4 µg or 1 µg	Injection (+thrombin)	Collage Graft o
2018/ Wang et al.[39]	Rabbit	48	Θ 2.5	LDE, length 30	Fem.: I/S Tib.: soft tissue	I/S MgZnSr Θ 3 x 8 mm length	No	Fem. fixation: I/S	I/S (PL
2019 / Fu et al.[30]	Dog	21	Θ 4	FDL, Θ 4, length 40	Implant in Fem + Tib. tunnels	Alloy ZK60 Mg Bio-Transfix (12 x 2 mm) porous, resorbable	BMP2	Fixation with implant	Implan BMP2/ Non-po implan
2019/ Han et al[35]	Rabbit	48	Θ 2.5	ST	Periosteum & soft tissues	Mb Nf Es PCL + multilayer + chitosan + Ac. Hyaluronic	SDF-1 + BMP2	Wrap-around graft	Mb PC PCL +
2019/ Zhang et al.[21]	Rabbit	18	Θ 2.5	ST	T/O suture (bone bridge) + soft tissue	Gelatin sponge 5 x 5 x 2 mm	PRP (1 mL)	Wrap-around graft	Sponge PRP
2020/ Sun et al.[40]	Rabbit	60	Θ 2.5	LDE, length 30	Fem.: I/S studied Tib.: T/O suture	I/S alloy ZnMnMg (Θ 1.9-3 x 8 mm length)	None	Fem. graft fixation with I/S	I/S Ti
2020/ Wei et al[34]	Rabbit	60	Θ 2 (w/o sponge) 2.5 Θ (w/ sponge)	Achilles Θ 1.3–1.6	T/O suture	Collagen sponge (10 x 5 mm)	Solution OPG (1 mg) + BMP2 (1 µg): (100 µg/mL)	Wrap-around graft	Graft o sponge OPG + BMP2

Fem.: femoral; Tib: tibial; UN: unknown; LDE: long digital extensor; ST: Semitendinosus, FDL: flexor digitorum longus; FDS: flexor digitorum superficialis; LCL: lateral collateral ligament; MCL: medial collateral ligament; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; PGA: propylene glycol alginate; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor

Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor; SDF 1: stromal cell-derived factor 1; PBS: phosphate buffered saline; ctrl: control group; T/O: transosseous * femoral and tibial tunnel identical unless specified

Table 3: Main quantitative histological results of the included studies

Year / Author Animal/ Strategy	Study design	Histological analysis methods	Results vs. controls at each t		
			Before 2 weeks	2-3 weeks	4-6 weeks
2004/Mihelic et al.[36] Sheep/ Wrap-around	Collagen + BMP7 vs Collagen	Histomorphometry: Bone volume (B.V., %) trabecular thickness (T.T., µm), number (T.N. per mm), Separation (T.S., µm), Tendon fiber outgrowth (T.F.O., µm)		**B.V.:** **Collagen+BMP7: 41,5** **Collagen: 30,6** **(p< 0,05)** T.T.: N.S. (119,5-131,6) **T.N.:** **Collagen+BMP7: 3,2** **Collagen: 2,6** **(p< 0,05)** **T.S.:** **Collagen+BMP7: 189** **Collagen:** **277,4** **(p< 0,05)** **T.F.O.: Collagen+BMP7: 995** **Collagen: 486** **(p< 0,01)**	
2008/ Sasaki et al.[22] Dog/ Wrap-around	Gelatin + G-CSF vs Gelatin	Quantitative Immunohistology (number of capillaries / fields of view)		**Gelatin+G-CSF: 781,5** **Gelatin:** **316,5 (p <0,01)**	
2013/Oka et al.[37] Rabbit/ Wrap-around	Gelatin + Simvastatin vs Gelatin	Quantitative Immunohistology (number of capillaries and osteoblasts / mm²)		***Capillaries*** **Gelatin + Simvastatin: 112** **Gelatin: 72 (p<0,01)** ***Osteoblasts*:** **Gelatin+Simvastatin: 495** **Gelatin: 272 (p<0,001)**	
2019/ Zhang et al.[21] Rabbit/ Wrap-around	Gelatin+PRP vs PRP vs Gelatin	Semi-quantitative score (Tan et al. / 10)			
2020/ Wei et al[34] Rabbit/ Wrap-around	Collagen +OPG + BMP2 vs Collagen vs OPG + BMP2 vs Graft	Histomorphometry: Tunnels Enlargement (mm) New bone area (mm²)			*Tunnels enlargement*: N.S. vs graft *NB area:* N.S. vs graft
		Yamakado et al. score (four items scored from 0 to 3)			Better in collagen + OPG + BMP2 group (no stats)
2005/ Yamazaki et al.[27] Dogs/ Injection	Fibrin (tib.) + TGF-B1 vs fibrin vs Graft	Quantitative: Bone ingrowth in tunnel (%)		Fibrin + TGF-B1: 55-65% Graft: 30-40% (no stats)	
2007/ Ma et al.[41] Rabbit/ Injection	CPM + BMP2 vs CPM + Noggin vs CPM	Histomorphometry: New bone ingrowth (mm)		**CPM+BMP2 :0,30 81% > control (CPM) (p<0,05)**	**CPM+BMP2: 0,32: 89% > control (CPM) (p<0,05)**

2007/ Rodeo et al.[18] Rabbit/ Injection	CPM + OPG vs CPM + RANKL vs CPM vs Graft	Histomorphometry: New bone ingrowth (mm)	**CPM + OPG: 0,19 (p=0,004)** **Control (CPM): 0,1**	N.S. (0,12-0,19)
		Immunohistology (number of osteoclasts / mm of tunnel)	**CPM+OPG: 1 / mm (p = 0,014)** **Control (CPM): 5 / mm**	**CPM+OPG: 4 / mm (p = 0,02)** **Control (CPM): 8/ mm**
2008/ Gulotta et al.[19] Rabbit/ Injection	Mg + Ca + P Glue vs Graft	Histomorphometry: Interface width (µm)	N.S. (145-190 fem, 145-149 tib)	***Fem.:*** **Mg+Ca+P: 70 (p=0,04)** **Graft: 157** ***Tib.:*** **Mg+Ca+P: 76 (p=0,04)** **Graft: 150**
2009/ Wen et al[32] Rabbit/ Injection	BCPC vs Graft	Quantitative: Fluorescence: new bone formation (µm/week)		N.S. (17-19)
2011/ Pan et al.[33] Rabbit/ Injection	CPC + BMP vs Fibrin + BMP vs Graft	Quantitative Fluorescence: bone mineralization rate (µm / day)		**CPC+BMP: 2,9** **Graft: 2,3** **(p<0,05)**
2014/ Kuang et al.[24] Rabbit/ Injection	Sr-CPC vs CPC	Score Yeh et al. (new bone, FC, graft connection, each from 0 to 3, total from 0 to 9)	**CPC: 1,2** **Sr-CPC: 1,9 (p<0,001)**	**CPC: 2** **Sr-CPC: 3,3 (p<0,001)**
2007/Walsh et al.[17] Sheep / Tib. Graft fixation	PLC I/S vs PLLA I/S	Semi-quantitative score: new bone ingrowth (0 to 4)		N.S.
2015/Cheng et al.[25] Rabbit/ Fem. Graft Fixation	Mg I/S vs Ti I/S	Semi quantitative (FC interface, %)	N.S.	N.S.
		Immunohistology (BMP2 and VEGF detection)		**BMP2: Mg > Ti (p<0,05)**
		Score Kuang et al. (3 items from 0 to 3, total: 0 to 9)	N.S.	N. S
2011/Mutsuzaki et al[28] Sheep/ Immersion	Ca + P solutions vs Graft	Quantitative Cartilage formation (%)		
		Number of osteoclasts / mm		
2016/Mutsuzaki et al.[26] Sheep/ Immersion	Ca + P solutions vs Graft	Histomorphometry: Cartilage area (mm^2) Nonbonding gap in BTI (mm)		

score Murray et al. (cells, extracellular matrix and vascular characteristics, total /28)

Fem.: femoral; Tib: tibial; FC : fibrocartilage; NB : new bone; SF : Sharpey fibers ; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor; SDF 1: stromal cell-derived factor 1; VEGF: Vascular Endothelial Growth Factor ; N.S.: not significant; BTI: bone-tendon interface;* Mean results. Significative results were written in bold.

Table 4: Main biomechanical results of the included studies

Year / Author Animal/ Strategy	Study design Strain rate	Biomechanical analysis methods	Results at each time-point*				
			Before 2 weeks	2-3 weeks	4-6 weeks	8 weeks	12 weeks and after
2001/ Anderson et al.[16] Rabbit/Wrap-around	Collagen +BMP vs Collagen vs <u>Graft</u> 40 mm/sec	UFL (N)		**Collagen + BMP: 54,7** **Graft: 37,3** **(p = 0,04)**	**Collagen + BMP: 65,8** **Graft: 39,4** **(p = 0,01)**	**Collagen + BMP: 70,7** **Graft: 39,4** **(p < 0,001)**	
		Tunnel failure (%)		Collagen + BMP: 85% Graft: 92% N.S.	Collagen + BMP: 69% Graft: 54% N.S.	Collagen + BMP: 55% Graft: 31% N.S.	
2004/Miheli c et al.[36] Sheep /Wrap-around	Collagen + BMP7 vs <u>Collagen</u> 0,1N / sec	UFL (N)		**Collagen + BMP: 350** **Collagen: 212** **(p< 0,01)**	**Collagen + BMP: 380** **Collagen: 215** **(p< 0,01)**		
2008/ Sasaki et al.[22] Dog/Wrap-around	Gelatin + G-CSF vs <u>Gelatin</u> 20mm / min	UFL (N)		N.S. (25,5-27,2)	**Gelatin + G-CSF: 99,5** **Gelatin: 32** **(p< 0,01)**		
		Stiffness (N/mm)		Gelatin+G-CSF 19,6 Gelatin: 15,2 (no stats)	Gelatin+G-CSF 25,5 Gelatin 11,9 (no stats)		
		Tunnel failure (%)		Both groups: 100 %	Gelatin+G-CSF: 16,7% Gelatin: 100%		
2013/Oka et al.[37] Rabbit/Wrap-around	Gelatin + Simvastatin vs <u>Gelatin</u> 10mm / min	UFL (N)		**Gelatin+ Simvastatin: 32,5** **Gelatin: 21,6** **(p<0,05)**	N.S. (33,4-33,5)	N.S. (38,4-36,7)	
		Stiffness (N/mm)		N.S. (8,9-12,8)	N.S. (11,1-13,6)	N.S. (15,3-16,3)	
		Tunnel failure (%)		Both groups: 100%	Both groups: 33%	Both groups: 33%	

2015/Han et al[38] Rabbit/Wrap-around	Mb PCL / nano-HA/ Collagen vs Graft 2 mm / min	UFL (N) Stiffness (N/mm) Tunnel failure (%)			N.S. (26-28) N.S. (7-8) Both groups: 100%	**Mb PCL: 58,4 Graft: 39,9 (p<0,001)** **Mb PCL: 15,2 Graft: 10,2 (p<0,001)** Both groups: 100%
2019/ Han et al[35] Rabbit/Wrap-around	Mb multilayer + SDF1 + BMP2 vs Mb PCL + BMP2 vs Mb PCL 5 mm / min	UFL (N) Stiffness (N/mm)			N.S. (16-31) N.S. (6-7)	**Mb multilayer + SDF1+BMP2: 79,9 Mb PCL: 63,5 (p<0,05)** **Mb multilayer + SDF1+BMP2: 19,5 Mb PCL: 10,8 (p<0,05)**
2019/ Zhang et al.[21] Rabbit/Wrap-around	Gelatin+PRP vs PRP vs Gelatin 20 mm / min	UFL (N) Stiffness (N/mm) Tunnel failure (%)				**Gelatin + PRP: 42,7 Gelatin: 36,9 (p=0,041)** **Gelatin + PRP: 3,2 Gelatin: 2 (p=0,017)** **All groups: 100%**
2004/ Tien et al.[42] Rabbit/Injection	CPC vs Graft 5 mm /second	UFL (N) Tunnel failure (%)	**At 1 week CPC: 6,5 Graft: 2 (p=0,027)** Both groups: 100%	**CPC: 11,5 Graft: 5,4 (p=0,028)** Both groups: 100%		
2005/ Yamazaki et al.[27] Dogs/Injection	Fibrin (tib.) + TGF-B1 vs fibrin vs Graft 20 mm /min	UFL (N) Stiffness (N/mm)		**Fibrin+TGF-B1: 188,2 Graft: 87,4 (p=0,003)** **Fibrin+TGF-B1: 72 Graft: 33 (p=0,002)**		

		Tunnel failure (%)	All groups: 100%			
2007 / Huangfu et al.[29]	TCP vs Graft	UFL (N)	**TCP: 29,1 Graft: 14,4 (p<0,001)**	**4 weeks TCP: 62,9 Graft: 33,6 (p<0,001)** 6 weeks: N.S. (74,8-47,1)	No measured (midsubstance failures)	
Dogs /Injection	Strain 10 mm /min					
		Tunnel failure (%)	Both groups: 100%	TCP: 60% Graft: 80%	TCP: 40% Graft: 60%	TCP: 0% Graft: 20%
2007/ Ma et al.[41]	CPM + BMP2 vs	UFL (N)	N.S. (20-22)	N.S. (30-38)	N.S. (32-50)	
Rabbit/Injection	CPM + Noggin vs CPM 10 mm /min	Stiffness (N/mm)	N.S. (8-9)	N.S. (12-13)	**CPM + BMP2: 25 CPM: 11 (p< 0,05)**	
2007/ Rodeo et al.[18]	CPM + OPG vs	UFL (N)	N.S. (20-25)	N.S. (38)	N.S. (38-50)	
Rabbit/Injection	CPM + RANKL vs CPM vs Graft 10 mm /min	Stiffness (N/mm)	N.S. (8-10)	N.S. (11-14)	**CPM+ OPG: 22 CPM: 10 (p= 0,017)**	
		Tunnel failure (%)	All groups: 100%	Not described	CPM + OPG: 0% CPM: 100%	
2008/ Gulotta et al.[19]	Mg + Ca + P Glue vs Graft	UFL (N)	N.S. (36-37)	**Glue: 72 Graft: 43 (p=0,04)**		
Rabbit/Injection	10 mm/min	Tunnel failure (%)	Both groups: 100%	Both groups: 100%		
2009/ Wen et al[32]	BCPC vs Graft	UFL (N)		**BCPC: 94 Graft: 43 (p<0,05)**		**BCPC: 60 Graft: 39 (p<0,05)**
Rabbit/Injection	50 mm/min	Stiffness (N/mm)		**BCPC: 31 Graft: 15 (p<0,05)**		**BCPC: 22 Graft: 16 (p<0,05)**
		Tunnel failure (%)		**BCPC: 75% Graft: 100% (p=0,035)**		**BCPC: 37,5% Graft: 100% (p<0,013)**
2011/ Pan et al.[33]	CPC + BMP vs Fibrin + BMP vs	UFL (N)		**CPC+BMP: 79 Graft: 43 (p<0,01)**		N.S. (38-53)
Rabbit/Injection	Graft 50 mm/min	Tunnel failure (%)		CPC+BMP: 87,5% Graft: 100%		CPC+BMP: 37,5% Graft: 12,5%
2018/Lu et al.[23]	Collagen + FGF vs	UFL (N)		**Collagen+FGF: 25 Graft: 17 (p<0,05)**	N.S. (22-45)	**Collagen+FGF: 75 Graft: 32 (p<0,05)**
Rabbit/Injection	Collagen vs Graft					

	5 mm /min	Stiffness (N/mm)		**Collagen+F GF: 10 Graft: 5 (p<0,05)**	**Collagen+F GF: 7,5 Graft: 5 (p<0,05)**	**Collagen+F GF: 5 Graft: 4 (p<0,05)**
2007/Walsh et al.[17] Sheep/Tib. Graft Fixation	PLC I/S vs PLLA I/S 50 mm /min	UFL (N)		N.S. (50-60)	N.S. (210-220)	
		Tunnel failure (%)		Both groups: 0%	Both groups: 0%	
2015/Cheng et al.[25] Rabbit/ Fem. Graft Fixation	Mg I/S vs Ti I/S 0,5 mm /min	UFL (N)	Post-op N.S. (110-115)			N.S. (120-130)
		Stiffness (N/mm)	N.S. (25-27)			N.S. (45-55)
		Tunnel failure (%)	Both groups: 100%			Both groups: 0%
2018/ Wang et al.[39] Rabbit/ Fem. Graft Fixation	MgZnSr I/S vs PLA I/S 50 mm /min	UFL (N)		N.S. (38-40)		**MgZnSr: 68 PLA: 38 (p<0,05)**
2019 / Fu et al.[30] Dogs/Fixation	Porous Mg Bio-Transfix + BMP2 vs porous Mg BioTransfix vs non-porous Mg BioTransfix 1 mm /min	UFL (N)				**MgBioTransfix + BMP2: 251 MgBioTransfix: 177 Non-porous: 64 (p<0,05)**
2020/ Sun et al.[40] Rabbit/ Fem. Graft Fixation	ZnMnMg I/S vs Ti I/S 50 mm /min	UFL (N)		N.S. (50-75)		**12 weeks ZnMnMg: 110 Ti: 90 (p<0,05)** 16 weeks: N.S. (115)
		Tunnel failure (%)				12 weeks ZnMnMg: :0% Ti: 50%
2009/Mutsuzaki et al.[20] Sheep/ Immersion	Ca + P solutions vs Graft	UFL (N)		N.S. (109-117)		
		Stiffness (N/mm)		N.S. (28-32)		

	30 mm /sec	Tunnel failure (%)	CaP: 29% Graft: 43%		
2011/Mutsuzaki et al[28] Sheep/ Immersion	Ca + P solutions vs Graft	UFL (N)			At 26 weeks N.S. (562-575)
	30 mm /min	Stiffness (N/mm)			N.S. (43,5-50,5)
		Tunnel failure (%)			Both groups: 0%
2016/Mutsuzaki et al.[26] Sheep /Immersion	Ca + P solutions vs Graft	Anterior tibial translation at 50N load (mm) Internal rotation (degree) at 2N/m torque at 0°,60°, 90° knee flexion			At 26 weeks Anterior tib. Translation N.S. Internal tibial torque: N.S.

Fem.: femoral; Tib: tibial; FC : fibrocartilage; NB : new bone; SF : Sharpey fibers ; UFL : Ultimate Failure Load; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor; SDF 1: stromal cell-derived factor 1; VEGF: Vascular Endothelial Growth Factor ; N.S.: not significant;* Mean results. Significant results are written in bold. When not significant, results were reported as minimal and maximal values between groups, in brackets.

Table 5: Main radiological results of the included studies

Year / Author Animal/ Strategy	Study design	Radiological analysis	Results vs. controls at each time-point*			
			2-3 weeks	4-6 weeks	8 weeks	12 weeks and after
2001/ Anderson et al.[16] Rabbit /Wrap-around	Collagen +BMP vs Collagen vs Graft	Qualitative MRI: NB	NB in 21 tunnels (no comparison)			
2008/ Sasaki et al.[22] Dog/Wrap-around	Gelatin + G-CSF vs Gelatin	CT: bone tunnel area (mm²)	N.S. (25,6-29,9)	**Gelatin+G-CSF: 21,51 Gelatin: 41,8 (p< 0,05)**		
2013/Oka et al.[37] Rabbit/Wrap-around	Gelatin + Simvastatin vs Gelatin	Micro-CT: bone tunnel area (mm²)	**Gelatin+Simvastatin: 3,25 Gelatin: 4,13 (p<0,05)**	**Gelatin+Simvastatin: 1,61 Gelatin: 2,71 (p<0,01)**	**Gelatin+Simvastatin: 0,94 Gelatin: 1,61 (p=0,06)**	
2019/ Zhang et al.[21] Rabbit/Wrap-around	Gelatin+ PRP vs PRP vs Gelatin	Qualitative MRI			Gelatin: No NB, no FC Gelatin+PRP: presence of FC	

2007/ Ma et al.[41] Rabbit/Injection	CPM + BMP2 vs CPM + Noggin vs CPM	Radiographs : tunnel diameter (mm)	**CPM + BMP2: 1,9 15% smaller than CPM (p<0,05)**	**CPM + BMP2: 1,6 25% smaller than CPM (p<0,05)**	**CPM + BMP2: 1,3 42% smaller than CPM (p<0,05)**	
2007/ Rodeo et al.[18] Rabbit/Injection	CPM + OPG vs CPM + RANKL vs CPM vs Graft	Radiographs: Bone tunnel area (mm^2)	N.S. (3,2-4,2)	N.S. (3,4-4,3)	N.S. (2,2-3,6)	
2008/ Gulotta et al.[19] Rabbit/Injection	Mg + Ca + P Glue vs Graft	Micro-CT: Total Bone Volume (mm3) Bone/Tissue Ratio Bone Mineral Tissue Mineral Trabecular Thickness (µm)	All measures N.S. (no data)	Bone Volume: Glue: 27 Graft: 12 (p=0,003, fem.) (N.S. tib.) All other measures N.S. (no data)		
2009/ Wen et al[32] Rabbit/Injection	BCPC vs Graft	Micro-CT: Bone/Tissue Volume Ratio (BV/TV) Trabecular Thickness (TT, mm)		BV/TV: Tib.: **BCPC: 0,084 Graft:0,045 (p<0,05)** Fem: N.S. TT: N.S.		BV/TV: **Tib. /Fem. BCPC: 0,087 / 0,144 Graft:0,060 / 0,064 (p<0,05)** TT: N.S.
2011/ Pan et al.[33] Rabbit/Injection	CPC + BMP vs Fibrin + BMP vs Graft	Micro-CT: Bone mineral density (mg/cm^3)	**CPC+BMP: 93 Graft: 69 (p<0,05)**			N.S. (109-125)
2007/Walsh et al.[17] Sheep /Tib. Graft fixation	PLC I/S vs PLLA I/S	Qualitative CT		PLC: NB, yes PLLA: NB, no		At 12 weeks PLC: NB, yes PLLA: NB, no At 26 and 52 weeks PLC screw undetectable, replaced by NB

						PLLA screw intact, limited NB
2015/Cheng et al.[25] Rabbit /Fem. Graft fixation	Mg I/S vs Ti I/S	Qualitative micro-CT				Mg screw: corrosion + mineral deposition Ti screw: intact
2018/ Wang et al.[39] Rabbit /Fem. Graft fixation	MgZnSr I/S vs PLA I/S	Micro-CT Bone volume (BV, mm3) Trabecular thickness (Tb.Th., mm), trabecular number, trabecular separation	**BV:** **MgZnSr: 3,3** **PLA: 1,75** **(p<0,05)** **Tb. Th:** **MgZnSr: 0,27** **PLA: 0,17** **(p<0,05)**		**At 12 weeks** **BV: MgZnSr: 3,2** **PLA: 1,4** **(p<0,05)** **Tb.Th.: MgZnSr: 0,32** **PLA: 0,17** **(p<0,05)**	At 16 weeks: MgZnSr: Replaced by NB **BV: MgZnSr: 2,9** **PLA: 1,2** **(p<0,05)** **Tb.Th.: MgZnSr:0,26** **PLA: 0,16** **(p<0,05)**
2019 / Fu et al.[30] Dogs/Fixation with implant	Porous Mg Bio-Transfix + BMP2 vs porous Mg BioTransfix vs non-porous Mg BioTransfix	Qualitative X-Ray, MRI, Micro-CT	MgBioTransfix + BMP2: NB, yes	MgBioTransfix + BMP2: NB, yes, > controls	MgBioTransfix+BMP2: replaced by NB, NB formation: N.S.	
2020/ Sun et al.[40] Rabbit/Fem. graft fixation with I/S	ZnMnMg I/S vs Ti I/S	Micro-CT: Bone volume/ Total volume ratio (BV/TV) Trabecular number	**BV/TV:** **ZnMnMg: 0,6** **Ti: 0,5** **(p<0,05)** **TbN:** **ZnMnMg: 3,1** **Ti: 2,9** **(p<0,05)**		At 12 weeks: N.S. At 16 weeks: N.S.	

		(TbN / mm)	
2011/Mutsuzaki et al[28] Sheep/Immersion	Ca + P solutions vs Graft	Micro-CT: Tunnel diameter (mm) Tunnel area (mm²) Tunnel enlargement (%)	At 26 weeks: Tunnel enlargement: Fem. CaP: 118% Graft: 170% (p=0,027) Tib. N.S. (66-114%)
2016/Mutsuzaki et al.[26] Sheep/Immersion	Ca + P solutions vs Graft	Micro-CT Tunnel area (mm²) Tunnel enlargement (%)	At 26 weeks Tunnel enlargement: N.S. for fem. (28-51%) and tib. (-2,8 – 2,9%)

Fem.: femoral; Tib: tibial; FC: fibrocartilage; NB: new bone; SF: Sharpey fibers; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; CPM: Calcium phosphate matrix; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; 1; N.S.: not significant; * Mean results. Significant results are written in bold. When not significant, results were reported as minimal and maximal values between groups, in brackets.

Appendix 1 : Research Strategy

PubMed (filter English and French) (May 2021) :

("Tendon-bone"[Title/Abstract]) AND ("histology"[Title/Abstract])
("tendon-bone"[Title/Abstract]) AND (histology)
("Tendon-bone"[Title/Abstract]) AND ("tissue engineering"[Title/Abstract])
("Tendon-bone"[Title/Abstract]) AND ("in vivo"[Title/Abstract])
("Tendon-bone"[Title/Abstract]) AND ("animal model"[Title/Abstract])
("Tendon-bone"[Title/Abstract]) AND ("model"[Title/Abstract])
("Tendon-bone"[Title/Abstract]) AND ("scaffold"[Title/Abstract])
("tendon-bone"[Title/Abstract]) AND ("interface"[Title/Abstract])
("tendon-bone"[Title/Abstract]) AND ("healing"[Title/Abstract])
("tendon-bone"[Title/Abstract]) AND ("repair"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("interface"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("histology"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("tissue engineering"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("in vivo"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("model"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("animal model"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("scaffold"[Title/Abstract])
("tendon graft-bone"[Title/Abstract]) AND ("healing"[Title/Abstract])
(« anterior cruciate ligament reconstruction" and "model")

Embase (May 2021)

'tendon bone':ti,ab,kw AND histology AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'tissue engineering' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'in vivo' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'animal' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'animal model' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'scaffold' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'interface' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'healing' AND ([english]/lim OR [french]/lim)
'tendon bone':ti,ab,kw AND 'repair' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'interface' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'histology' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'tissue engineering' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'in vivo' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'model' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'animal' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'scaffold' AND ([english]/lim OR [french]/lim)
'tendon graft-bone':ti,ab,kw AND 'healing' AND ([english]/lim OR [french]/lim)
'anterior cruciate ligament reconstruction':ti,ab,kw AND **model**:ti,ab,kw AND ([embase]/lim OR [pubmed-not-medline]/lim) AND ([article]/lim OR [article in press]/lim OR [data papers]/lim) AND ([english]/lim OR [french]/lim) AND [animal model]/lim AND [animals]/lim

Web of Science (May 2021) :

All databases
All years 1950-2021
Advanced search and topic
Language = english

1) AB=(tendon AND bone) AND AB=(histology) AND TS=(knee OR knees)
2) AB=(tendon AND bone) AND AB=(tissue engineering) AND TS=(knee OR knees)
3) AB=(tendon AND bone) AND AB=(in vivo) AND TS=(knee OR knees)
4) AB=(tendon AND bone) AND AB=(animal) AND TS=(knee OR knees)

5) AB=(tendon AND bone) AND AB=(animal model) AND TS=(knee OR knees)
6) AB=(tendon AND bone) AND AB=(scaffold) AND TS=(knee OR knees)
7) AB=(tendon AND bone) AND AB=(interface) AND TS=(knee OR knees)
8) AB=(tendon AND bone) AND AB=(healing) AND TS=(knee OR knees)
9) AB=(tendon AND bone) AND AB=(repair) AND TS=(knee OR knees)
10) AB=(tendon graft AND bone) AND AB=(interface) AND TS=(knee OR knees)
11) AB=(tendon graft AND bone) AND AB=(histology) AND TS=(knee OR knees)
12) AB=(tendon graft AND bone) AND AB=(tissue engineering) AND TS=(knee OR knees)

13) AB=(tendon graft AND bone) AND AB=(in vivo) AND TS=(knee OR knees)
14) AB=(tendon graft AND bone) AND AB=(animal) AND TS=(knee OR knees)
15) AB=(tendon graft AND bone) AND AB=(animal model) AND TS=(knee OR knees)
16) AB=(tendon graft AND bone) AND AB=(scaffold) AND TS=(knee OR knees)
17) AB=(tendon graft AND bone) AND AB=(healing) AND TS=(knee OR knees)
18) AB=(tendon graft AND bone) AND AB=(repair) AND TS=(knee OR knees)
« anterior cruciate ligament reconstruction" and "model" (abstract)

Appendix 2: Qualitative and quantitative histological results of the included studies +

Year / Author Animal/ Strategy	Study design	Histological analysis methods	Results vs. controls at each time-point*				
			Before 2 weeks	2-3 weeks	4-6 weeks	8 weeks	12 weeks and after
2001/ Anderson et al.[16] Rabbit/Wrap-around	Collagen +BMP vs Collagen vs Graft	Qualitative		Collagen + BMP: Fibrovascular tissue FC: yes NB: yes Graft: Fibrovascular tissue FC: yes NB: yes, rare	Collagen + BMP: FC: yes SF: yes > controls Graft: FC: yes	Collagen + BMP: FC, yes > controls NB, yes > controls Graft: heterogenous healing	
2004/Mihelic et al.[36] Sheep/ Wrap-around	Collagen + BMP7 vs Collagen	Qualitative Histomorphometry: Bone volume (B.V., %) trabecular thickness (T.T., µm), number (T.N. per mm), Separation (T.S., µm), Tendon fiber outgrowth (T.F.O., µm)		Collagen + BMP: NB: yes, > controls FC: No, in both groups **B.V.:** **Collagen+BMP7 : 41,5** **Collagen: 30,6** **(p< 0,05)** T.T.: N.S. (119,5-131,6) **T.N.:** **Collagen+BMP7 : 3,2** **Collagen: 2,6** **(p< 0,05)** **T.S.:** **Collagen+BMP7 : 189** **Collagen: 277,4** **(p< 0,05)** **T.F.O.:** **Collagen+BMP7 : 995** **Collagen: 486** **(p< 0,01)**			
2008/ Sasaki et al.[22] Dog/ Wrap-around	Gelatin + G-CSF vs Gelatin	Quantitative Immunohistology (number of capillaries / field of view)		**Gelatin+G-CSF: 781,5** **Gelatin: 316,5 (p <0,01)**			

2013/Oka et al.[37] Rabbit/ Wrap-around	Gelatin + Simvastatin vs Gelatin	Qualitative	Gelatin+Simvastatin: connective tissue, NB: yes, > control FC: yes, Gelatin: fibrous tissue NB: yes, FC: no	Both groups: connective tissue, NB: yes Gelatin+Simvastatin: SF: yes > control	Gelatin+Simvastatin: FC: yes, > control	
		Quantitative Immunohistology (number of capillaries and osteoblasts / mm²)	**Capillaries** **Gelatin + Simvastatin: 112** **Gelatin: 72 (p<0,01)** **Osteoblasts:** **Gelatin+Simvastatin: 495** **Gelatin: 272 (p<0,001)**			
2015/Han et al[38] Rabbit/ Wrap-around	Mb PCL / nano-HA/ Collagen vs Graft	Qualitative		Both groups: Fibrous tissue	Both groups: NB: yes SF: yes Mb PCL/nano-HA/Collagen: Fibrous tissue narrower than graft group	
2019/ Han et al[35] Rabbit/ Wrap-around	Mb multilayer + SDF1 + BMP2 vs Mb PCL + BMP2 vs <u>Mb PCL</u>	Qualitative Immunohistology (OCN, OPN)		All groups: Fibrous tissue Mb multilayer+ SDF1+BMP2: More OCN and OPN than controls	Mb multilayer + SDF1+BMP2: NB: yes, > than controls More OCN and OPN than controls	
2019/ Zhang et al.[21] Rabbit/ Wrap-around	Gelatin+PRP vs PRP vs <u>Gelatin</u>	Qualitative			PRP groups: FC: yes SF: yes NB: yes Gelatin: Fibrovascular tissue	
		Semi-quantitative score (Tan et al. / 10)			**Gelatin+PRP: 7,83** **PRP: 6,17 (p = 0,039)** **Gelatin: 5,17 (p=0,003)**	
2020/ Wei et al[34] Rabbit/ Wrap-around	Collagen +OPG + BMP2 vs Collagen vs OPG + BMP2 vs <u>Graft</u>	Qualitative		Collagen + OPG+BMP2: fibrovascular tissue NB: yes All controls: Fibrovascular tissue	Collagen + OPG+BMP2: Fibrovascular tissue FC: yes All controls: lower blood vessels Sharpey fibers: yes NB: yes	Collagen + OPG+BMP2 FC: yes, > controls All controls: SF: yes NB: yes FC: yes
		Histomorphometry: Tunnels Enlargement (mm) New bone area (mm²)		*Tunnels enlargement*: N.S. vs graft *NB area:* N.S. vs graft	***Tunnels enlargement:*** **Collagen + OPG+BMP2: 0,45 (fem), 0,42 (tib)** **Graft: 0,70 (fem) (p<0,01),**	***Tunnels enlargement :*** **Collagen + OPG+BMP2 : 0,44 (fem), 0,41 (tib)**

						0,80 (tib) (p<0,01) ***NB area:*** **Collagen + OPG+BMP2: 0,38 Graft: 0,21 (p<0,01)**	**Graft: 0,80 (fem (p<0,01), 0,87 (tib) (p< 0,01)** ***NB area:*** **Collagen + OPG+BMP 2: 0,45 Graft: 0,32 (p < 0,01)**
		Yamakado et al. score (four items scored from 0 to 3)			Better in collagen + OPG + BMP2 group (no stats)	Better in Collagen + OPG+BMP2 group (no stats)	Better in Collagen + OPG+BMP2 group (no stats)
2004/ Tien et al.[42] Rabbit/ Injection	CPC vs Graft	Qualitative	At 1 week CPC: fibrous tissue FC: no SF: no Graft: fibrous tissue	CPC: NB: yes Graft Collagen fibers NB: no	CPC: NB, yes, anchoring with tendon fibers Graft: NB: no maturation of collagen fibers		At 12 weeks CPC: complete continuity between tendon fibers and NB Graft: NB: no At 24 weeks CPC: complete healing of interface Graft: No description
2005/ Yamazaki et al.[27] Dogs/ Injection	Fibrin (tib.) + TGF-B1 vs fibrin vs Graft	Qualitative Quantitative: Bone ingrowth in tunnel (%)		All groups: granulation tissue + NB Fibrin + TGF-B1: SF: yes, > controls Fibrin + TGF-B1: 55-65% Graft: 30-40% (no stats)			
2007 / Huangfu et al.[29] Dog/ Injection	TCP vs Graft	Qualitative		Both groups: fibrous tissue	At 4 weeks TCP: fibrous tissue NB: yes SF: yes Graft: fibrous tissue At 6 weeks: TCP: NB: yes, SF: yes Graft: connective tissue	TCP: NB: yes, SF: yes Graft: SF: yes	TCP: FC: yes Graft: FC: no SF: yes
2007/ Ma et al.[41] Rabbit/ Injection	CPM + BMP2 vs CPM + Noggin vs CPM	Histomorphometry: New bone ingrowth (mm)		**CPM+BMP2 :0,30 81% > control (CPM) (p<0,05)**	**CPM+BMP2: 0,32: 89% > control (CPM) (p<0,05)**	**CPM+BMP2 :0,31 113% > control (CPM) (p<0,05)**	
2007/ Rodeo et al.[18] Rabbit/ Injection	CPM + OPG vs CPM + RANKL	Histomorphometry: New bone ingrowth (mm)		**CPM + OPG: 0,19 (p=0,004) Control (CPM): 0,1**	N.S. (0,12-0,19)	**CPM + OPG: 0,2 (p= 0,033) Control (CPM): 0,11**	

	vs CPM vs Graft	Immunohistology (number of osteoclasts / mm of tunnel)	**CPM+OPG: 1 / mm (p = 0,014) Control (CPM): 5 / mm**	**CPM+OPG: 4 / mm (p = 0,02) Control (CPM): 8/ mm**	**CPM+OPG: 4 / mm (p > 0,05) Control (CPM): 4/ mm**	
2008/ Gulotta et al.[19] Rabbit/ Injection	Mg + Ca + P Glue vs Graft	Qualitative	Both groups: fibrovascular tissue, collagen fibers,	Both: fibrovascular tissue, collagen fibers		
		Histomorphometry: Interface width (µm)	Mg + Ca + P: FC: yes, > graft N.S. (145-190 fem, 145-149 tib)	Mg + Ca + P: FC: yes, > graft ***Fem.:*** **Mg+Ca+P: 70 (p=0,04) Graft: 157** ***Tib.:*** **Mg+Ca+P: 76 (p=0,04) Graft: 150**		
2009/ Wen et al[32] Rabbit/ Injection	BCPC vs Graft	Qualitative		BCPC: SF: yes		BCPC: direct connection
		Quantitative: Fluorescence: new bone formation (µm/week)		N.S. (17-19)		N.S. (no data)
2011/ Pan et al.[33] Rabbit/ Injection	CPC + BMP vs Fibrin + BMP vs <u>Graft</u>	Qualitative	All groups: fibrovascular tissue, CPC+BMP: NB: yes Fibrin+BMP: FC: yes	CPC+BMP: NB: yes SF: yes Fibrin+BMP: FC: yes Control: fibrovascular tissue		CPC+BMP: NB: yes FC: yes SF: yes Fibrin+BMP: NB, yes SF, yes Control: SF: yes
		Quantitative Fluorescence: bone mineralization rate (µm / day)		**CPC+BMP: 2,9 (p<0,05) Graft: 2,3**		**CPC+BMP: 3 (p<0,05) Graft: 2,1**
2014/ Kuang et al.[24] Rabbit/ Injection	Sr-CPC vs CPC	Semi-quantitative	Sr-CPC: NB: yes CPC: NB: no	Sr-CPC: NB: yes SF: yes CPC: NB: yes	Sr-CPC: SF: yes, > CPC CPC: SF, yes	At 12 weeks Sr-CPC: FC: yes CPC: SF: yes At 24 weeks Both groups: FC, yes
		Score Yeh et al. (new bone, FC, graft connection, each from 0 to 3, total from 0 to 9)	**CPC: 1,2 Sr-CPC: 1,9 (p<0,001)**	**CPC: 2 Sr-CPC: 3,3 (p<0,001)**	**CPC: 2,7 Sr-CPC: 4,6 (p<0,001)**	**12 weeks: Sr-CPC: 6,6 (p<0,001) CPC: 4,1** 24 weeks: N.S. (6,7-6,8)
2018/Lu et al.[23] Rabbit/ Injection	Collagen + FGF vs Collagen vs Graft	Qualitative		All groups: fibrovascular tissue, SF: yes	Collagen+FGF: NB: yes, > graft FC: yes, > graft SF: yes > graft	Collagen+FGF: FC: yes, > graft SF, yes, > graft

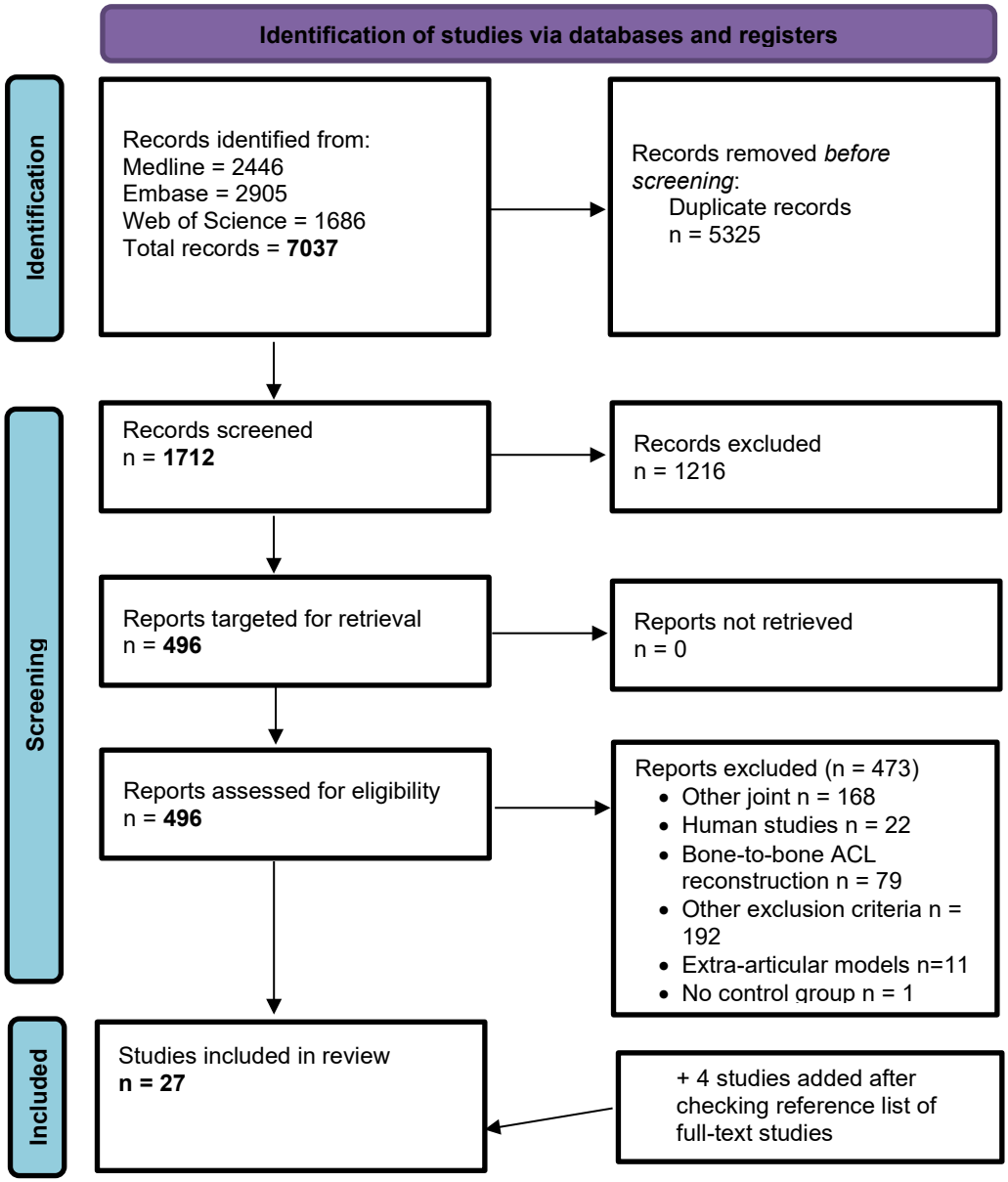
2007/Walsh et al.[17] Sheep / Tib. Graft fixation	PLC I/S vs PLLA I/S	Qualitative			Both groups: SF: yes NB: yes		At 12 weeks Both groups: SF: yes NB: yes At 26 and 52 weeks: PLC: not found, replaced by new bone PLLA: intact
		Semi-quantitative score: new bone ingrowth (0 to 4)			N.S.		**12 weeks: PLC: 1,5/4 PLLA = 0/4 (p<0,05)** **26 weeks: PLC: 3,5/4 PLLA: 0/4 (p<0,05)** **52 weeks: PLC: 4/4 (p<0,05) PLLA: 0/4**
2015/Cheng et al.[25] Rabbit/ Fem. Graft Fixation	Mg I/S vs Ti I/S	Semi quantitative (FC interface, %)		N.S.	N.S.	**Mg :35% (p<0,05) Ti: 22%**	**Mg: 60% (p<0,01) Ti: 40%**
		Immunohistology (BMP2 and VEGF detection)			**BMP2: Mg > Ti (p<0,05)**		N.S.
		Score Kuang et al. (3 items from 0 to 3, total: 0 to 9)		N.S.	N.S.	**Mg: 5,1 (p<0,05) Ti: 3,7**	**Mg: score 7/9 (p<0,05) Control (Ti): 5,3**
2018/ Wang et al.[39] Rabbit/ Fem. Fixation	MgZnSr I/S vs PLA I/S	Qualitative, Fluorescence			MgZnSr: more bone than PLA		At 16 weeks MgZnSr: completely degraded, replaced by new bone, more bone than PLA
2019 / Fu et al.[30] Dogs/ Fixation	Porous Mg Bio-Transfix + BMP2 vs porous Mg BioTransfix vs non-porous Mg BioTransfix	Qualitative					MgBioTransfix: Replaced by new bone
2020/ Sun et al.[40] Rabbit/ Fem. Graft Fixation	ZnMnMg I/S vs Ti I/S	Qualitative Fluorescence			At 6 weeks: ZnMnMg: NB: yes Ti: NB: no		At 12 and 16 weeks ZnMnMg: NB: yes, > Ti
2004/Mutsuzaki et al.[31] Rabbit/ Immersion	Ca + P solutions vs Graft	Qualitative	At 3 days: Both groups: fibrin clot	At 2 weeks: CaP: NB: yes FC: yes Graft: fibrous tissue	At 4 weeks CaP: NB: yes, direct connections at interface		

			At 5 days: Both groups: fibrous tissue and bone trabeculae	At 3 weeks CaP: direct connections at interface Graft: fibrous tissue, no direct connections	Graft: SF: yes, indirect connection at interface	
2009/Mutsuzaki et al.[20] Sheep/Immersion	Ca + P solutions vs Graft	Qualitative			At 6 weeks: CaP: direct connection at interface Graft: fibrous tissue	
2011/Mutsuzaki et al[28] Sheep/ Immersion	Ca + P solutions vs Graft	Quantitative Cartilage formation (%)				At 26 weeks **CaP: 37,5% (p=0,0416)** **Graft: 8%**
		Number of osteoclasts / mm				***Fem.:*** **CaP: 0,29** **Graft: 1,68** **(p<0,05)** *Tib.:* N.S. (0,29-0,43)
2016/Mutsuzaki et al.[26] Sheep/ Immersion	Ca + P solutions vs Graft	Qualitative				At 26 weeks Both groups: SF: yes
		Histomorphometry: Cartilage area (mm²) Nonbonding gap in BTI (mm)				Cartilage area: CaP: 0,06 to 0,17 **(p=0,009, fem)** (N.S., tib) Graft: 0,02 to 0,28 Nonbonding gap: CaP: 0,6 to 1,1 Control: 1,5 to 3,1 (p=0,11, fem) **(p=0,047, tib.)**
		score Murray et al. (cells, extracellular matrix and vascular characteristics, total /28)				N.S. (20,8-22,8)

Fem.: femoral; Tib: tibial; FC : fibrocartilage; NB : new bone; SF : Sharpey fibers ; UN: unknown; I/S: Interference screw; HA: Hydroxyapatite; CPC: calcium phosphate cement; Ca: Calcium; P: Phosphorus; Ti: Titanium; Mg: Magnesium; Zn: Zinc; Mn: Manganese; PGA: propylene glycol alginate; CPM: Calcium phosphate matrix; TCP: Tricalcium phosphate; PRF: Platelet-rich fibrin; PRP: Platelet-rich plasma; BMP: Bone Morphogenetic Protein; TGF-B1: Transforming Growth Factor Beta-1; OPG: Osteoprotegerin; G-CSF: Granulocyte Colony-Stimulating Factor; FGF: fibroblast growth factor;

SDF 1: stromal cell-derived factor 1; VEGF: Vascular Endothelial Growth Factor ; N.S.: not significant; BTI: bone-tendon interface;* Mean results. When results were non-significant between groups, mean minimal and maximal results are indicated in brackets.

This appendix reports all histological analysis of the included studies. All qualitative observations were reported and when new bone (NB), Sharpey fibers (SF) or fibrocartilage (FC) were clearly searched in the studies, the results is indicated as "yes" in case of presence, "no" in case of absence of the tissue. If the authors indicated a difference between control groups, it was reported as ">" or "<" to control groups.



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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