■ SYSTEMATIC REVIEW

Biological augmentation of graft healing in anterior cruciate ligament reconstruction

A SYSTEMATIC REVIEW

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Aims

The success of anterior cruciate ligament reconstruction (ACLR) depends on osseointegration at the graft-tunnel interface and intra-articular ligamentization. Our aim was to conduct a systematic review of clinical and preclinical studies that evaluated biological augmentation of graft healing in ACLR.

Materials and Methods

In all, 1879 studies were identified across three databases. Following assessment against strict criteria, 112 studies were included (20 clinical studies; 92 animal studies).

Results

Seven categories of biological interventions were identified: growth factors, biomaterials, stem cells, gene therapy, autologous tissue, biophysical/environmental, and pharmaceuticals. The methodological quality of animal studies was moderate in 97%, but only 10% used clinically relevant outcome measures. The most interventions in clinical trials target the graft-tunnel interface and are applied intraoperatively. Platelet-rich plasma is the most studied intervention, but the clinical outcomes are mixed, and the methodological quality of studies was suboptimal. Other biological therapies investigated in clinical trials include: remnant-augmented ACLR; bone substitutes; calcium phosphate-hybridized grafts; extracorporeal shockwave therapy; and adult autologus non-cultivated stem cells.

Conclusion

There is extensive preclinical research supporting the use of biological therapies to augment ACLR. Further clinical studies that meet the minimum standards of reporting are required to determine whether emerging biological strategies will provide tangible benefits in patients undergoing ACLR.

Cite this article: *Bone Joint J* 2018;100-B:271–84.

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doi:10.1302/0301-620X.100B3.BJJ-2017-0733.R2 $2.00

Bone Joint J 2018;100-B:271–84.

Injury to the anterior cruciate ligament (ACL) is common, with an estimated annual incidence of 200 000 in the United States.[1] Anterior cruciate ligament reconstruction (ACLR) is the treatment of choice for young active patients,[2] with current evidence indicating autograft has superior outcomes compared with artificial ligament.[3,4] While the technique of ACLR has evolved,[5,6] it remains imperfect, with patients rarely returning to baseline function.[7] There is a risk of re-rupture and premature knee osteoarthritis.[8-11]The outcome of ACLR is thought to depend on the biological healing response referred to as "graft healing",[12] which collectively refers to osseointegration at the graft-tunnel interface and remodelling of the intra-articular graft, called ligamentization.

The native ACL inserts to bone through a direct fibrocartilaginous enthesis, which is characterized by a four-zone morphological structure with gradual transition from tendon to cartilage to mineralized cartilage to bone.[13] Following ACLR the graft heals with an indirect enthesis formed of biomechanically inferior fibrovascular scar tissue.[14] As a result, research is ongoing investigating interventions that can biologically augment graft healing after ACLR, and which may facilitate early aggressive rehabilitation and a faster return to physical activity.[15] Platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) are two promising biological therapies used in musculoskeletal research, but concerns about inadequate reporting of scientific details critical to outcome led to a recent expert

Table I. Criteria for assessing methodological quality of animal studies

Criteria	Score	Comments
Unit of sample	Unilateral 1/Bilateral 0	Unilateral operations are generally considered superior to bilateral operations
Standardization of surgical procedure	Yes 1/No 0	Details of graft harvest, surgical approach, drilling tunnels and fixation are provided
Description of surgical complications	Yes 1/No 0	Details such as general performance, perioperative morbidity and wound infection
Biomechanical testing reported	Yes 1/No 0	Mechanical testing is a useful outcome when assessing tendon-bone healing
Variation (ratio of standard deviation to the mean)	< 50%/ 1/> 50% 0	Large standard deviations indicate poor precision or large intra-group variability
Statistical method	Appropriate 1/Inappropriate 0	Appropriate statistical tests and measures of variation are used
Description of tendon-bone interface	Yes 1/No 0	Description of sampling of the region of interest is given during histological analyses
Semi-quantitative histological analysis	Yes 1/No 0	During histological analysis the use of scoring systems indicate better study quality

Table adapted from system first described by Fu et al[20]

consensus on the minimum reporting requirements for clinical studies evaluating PRP and MSCs.[16]

The objectives of this study were to: 1) perform a systematic review and categorize biological methods of augmentation of graft healing in ACLR; 2) to critically appraise studies against existing minimum reporting standards; 3) to identify the target site (graft-tunnel interface *versus* ligamentization) and timing (intraoperative *versus* postoperative) of biological interventions; and 4) to summarize progress of the different categories towards clinical translation and identify areas of future research.

Materials and Methods

We aimed to include all original studies evaluating biological interventions to augment graft healing after ACLR. We included studies using tendon autografts or synthetic ligament. We included clinical trials and animal studies of ACLR which used objective outcome measures of graft healing; these included animal studies reporting histological examination of the interface or clinical studies with radiological or clinical assessment of graft integrity. We excluded studies evaluating ACL repair, or treatment of partial ACL lesions or overstretch-injury. Animal studies reporting *in vitro* results without *in vivo* results, or those using extra-articular models of tendon-bone healing were excluded as were studies evaluating interventions without a biological component; studies focusing on augmentation of healing at the harvest site or surrounding muscles; studies without a control group or lacking adequate information for data extraction. If two studies were published using the same data the larger study was included.

A literature search of three databases (MEDLINE, EMBASE and Web of Science) was undertaken in February 2017 by one author (AH). No date of publication or language restriction was applied. Search strings were: ("Anterior Cruciate Ligament" OR ACL); AND ("Tendon-bone healing" OR "Biological therapy" OR"Stem cells" OR "Tissue Scaffolds" OR "Growth factor" OR "Bone morphogenic protein" OR "Platelet-rich plasma" OR "Calcium phosphate cement" OR "Bone substitutes" OR "Gene therapy"). Additional studies were added by screening bibliographies. Studies underwent title and abstract screening against inclusion/exclusion criteria and after initial screening, full manuscript review was undertaken. Data extraction and measurement of methodological quality was undertaken by two authors (AH and TT) using tools developed by the Cochrane collaboration. Randomized controlled trials were assessed using the Risk of Bias (RoB) tool[17] and non-randomized studies were assessed using the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) tool,[18] which both score risk of bias across seven domains. Minimum reporting standards[16] have been published for PRP (a 23 point checklist) and MSCs (a 25 point checklist); clinical studies evaluating PRP or MSCs were assessed to see how well they met the criteria.[16] The methodological quality of animal studies was assessed using an eight point scoring system[19,20] (Table I) and the quality of the outcome measures used in animal studies were ranked using a previously published scale on the basis of their relevance to clinical practice.[20] The ranks were: A, quantitative clinical outcome measures similar to those used in human trials; B, biomechanical testing of graft; C, quantitative biochemical measurements; D, semi-quantitative histological analysis or quantitative imaging; and E, simple qualitative histological or imaging analysis.

Results

Our search strategy identified 1879 studies. After removal of duplicates, 1422 studies underwent screening by title. Of

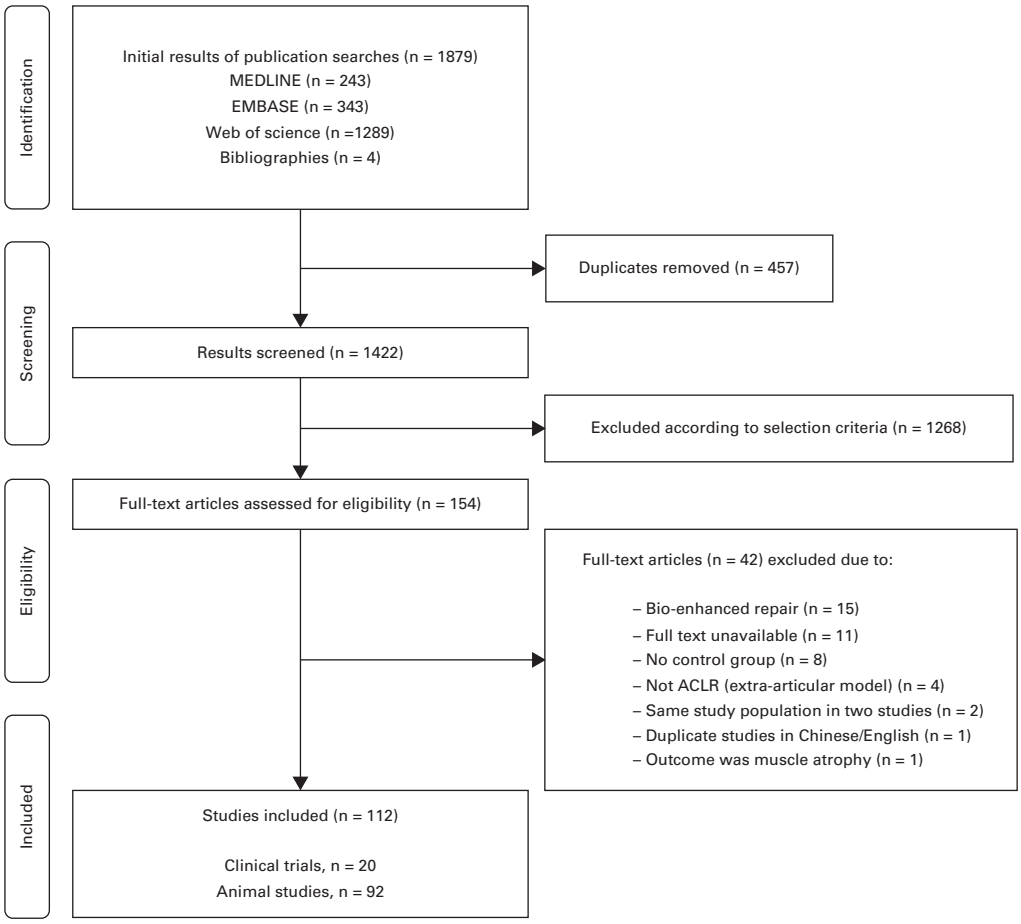

Fig. 1

A flowchart outlining the selection of studies for inclusion in this systematic review.

the 154 studies that underwent full manuscript review 42 studies did not meet the required criteria (Fig. 1). The remaining 112 studies (92 animal studies and 20 clinical trials) met the inclusion criteria (Tables II and III). The interventions studied fell into seven broad categories (Table IV).

Animal studies. The rabbit was the most common animal model (55 studies), followed by rodent (12 studies), sheep (eight studies), goat and pig (six studies each) and dog (five studies). The number of animals used ranged from six to 176, with a median of 36. Follow-up ranged from three weeks to 72 weeks, with a median of 12 weeks. Different ACLR graft types were used: the three most common were hamstring tendon (n = 25), flexor tendon of the lower limb (n = 18) and extensor tendon (n = 15). Two studies investigated both hamstring and flexor tendon grafts. A total of 15 studies used synthetic scaffolds; the two most common were silk scaffolds (eight studies) and polyethylene terephthalate (PET, seven studies). The grafts were investigated in one limb (unilaterally) in 45 studies and in both limbs (bilaterally) in 47 studies. The graft-tunnel interface was the target of biological intervention in 63 studies (68%), whereas ligamentization was the target in 20 studies; nine studies examined both sites. The most commonly investigated categories of biological intervention were biomaterials (29 studies) and growth factors (24 studies). The remaining categories were seen in the following frequencies: stem cells (13 studies), autologous tissue (ten studies), gene therapy (nine studies, of which seven concerned genetically modified stem cells), pharmaceuticals (six studies), and biophysical interventions (four studies). Seven studies combined different categories of intervention in the same study. The most common growth factors evaluated were bone morphogenic proteins (eight studies) and PRP (four studies). Of the animal studies, positive findings were seen in 86/92 studies (92%), negative findings in two (both investigating growth factors) and no difference in five (two studies of growth factors, two of autologous tissue, and one study of gene therapy).

Study quality. The methodological quality scores for animal studies ranged from 4/8 to 8/8 (Table II), with 89/92 of studies (97%) having a score of at least five points (considered to be the threshold for acceptable methodological quality). Excellent inter-rater reliability

Table II. Animal studies (n = 92) (see Table II continued on next page)

Author	Biological modulation	Category of intervention	Animal model	n	ACLR graft (uni/bi)	Site of action	Groups (n)	Follow-up min to max	Outcome measures	Finding	Evidence level	Quality score
Wang et al[66]	Mg-based interference screw	Biomaterial	Rabbit	112	Extensor (Bi)	Graft-bone interface	2	3 wks to 16 wks	Histology, biomechanical, imaging (pQCT)	Positive	B	6
Zhang et al[112]	Simvastatin	Pharmaceutical	Rabbit	36	PET (Uni)	Graft-bone interface	3	4 wks to 8 wks	Histology (IHC), biomechanical	Positive	B	6
Zhang et al[112]	ADSC - Runx2 transfected	Gene therapy (Stem cell)	Rabbit	30	Hamstring (Uni)	Graft-bone interface	3	2 wks to 52 wks	Histology, biomechanical, imaging (3D CT)	Positive	B	8
Teuschl et al[96]	ADSC - seeded silk scaffold	Stem Cell	Sheep	33	Silk (Uni)	Ligamentization	2	26 wks to 52 wks	Histology	Positive	E	5
Teng et al[21]	PRP, BMSC	Growth factor Stem Cell	Rabbit	30	Hamstring (Uni)	Graft-bone interface	3	8 wks	Histology, biomechanical, imaging (microCT)	Positive	B	6
Takahashi et al[124]	Augmented remnant repair	Autologous tissue	Sheep	42	Hamstring (Uni)	Ligamentization	2	4 wks to 12 wks	Laxity testing Histology, biomechanical	Positive	A	8
Song et al[125]	Augmented remnant repair	Autologous tissue	Rabbit	60	Hamstring (Uni)	Ligamentization	2	24 wks	Histology, biomechanical	No difference	B	8
Mutsuzaki 2016[86]	CaP-hybridised tendon	Biomaterial	Goat	10	Flexor (Uni)	Graft-bone interface	2	24 wks	Laxity testing, Histology, biomechanical, Imaging (CT)	Positive	A	7
Li et al[99]	BMSC-seeded silk scaffold	tem Cell	Rabbit	27	Silk (Bi)	Graft-bone interface	2	24 wks	Histology, biomechanical, imaging (microCT)	Positive	B	6
Kouroupis 2016	hAT-MSCs and hiPSC-MSC	Stem Cell	Pig	6	Bioscaffold (Uni)	Ligamentization	3	16 wks	Histology (IHC)	Positive	C	5
Kosaka et al[97]	ADSC	Stem Cell	Rabbit	80	Hamstring (Uni)	Graft-bone interface	2	2 wks to 12 wks	Histology, biomechanical	Positive	B	8
Kawakami[113]	ACL CD34+ - BMP2 transfected	Gene therapy (Stem cell)	Rat	48 (96)	Flexor (Bi)	Graft-bone interface	4	2 wks to 8 wks	Histology (IHC), biomechanical	Positive	B	5
Jin et al[61]	Hydroxyapatite-BMP coating	Biomaterial, Growth factor	Rabbit	24	Silk (Bi)	Graft-bone interface	4	1 wks to 3 wks	Histology (IHC)	Positive	C	5
Dai et al[131]	Periosteum patch	Autologous tissue	Goat	12	PET (Uni)	Graft-bone interface	2	52 wks	Histology, biomechanical, Imaging (CT, MRI)	Positive	B	7
Chou et al[68]	PLGA/collagen nanofiber and PLA bolt	Biomaterial	Rabbit	48	Extensor (Uni)	Graft-bone interface	2	8 wks to 16 wks	Histology, mechanical	Positive	B	5
Cheng et al[67]	Mg-based interference screw	Biomaterial	Rabbit	60	Hamstring (Uni)	Graft-bone interface	2	3 wks to 9 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	6
Chen et al[114]	BMSC - bFGF/BMP2 transfected	Gene therapy (Stem cell)	Rabbit	32	Hamstring (Bi)	Graft-bone interface	4	4 wks to 12 wks	Histology, biomechanical, imaging (microCT)	Positive	B	7
Takayama et al[115]	ACL CD34+ -VEGF transfected	Gene therapy (Stem cell)	Rat	60	Flexor (Bi)	Ligamentization	5	2 wks to 8 wks	Histology (IHC), biomechanical	No difference	B	6
Li et al[120]	Augmented remnant repair	Autologous tissue	Goat	12	PET (Bi)	Ligamentization	2	24 wks to 52 wks	Histology (IHC)	Positive	C	5
Leong et al[22]	bFGF	Growth factor	Rat	44	PCL (Uni)	Ligamentization	4	8 wks to 16 wks	Histology (IHC), biomechanical	Positive	B	8
Jang et al[101]	hUCB-MSCs	Stem cell	Rabbit	30	Hamstring (Uni)	Graft-bone interface	2	4 wks to 12 wks	Histology (IHC), imaging (microCT)	Positive	B	6
Hensler et al[23]	Fibrin clot	Growth factor	Goat	8	Achilles (Uni)	Ligamentization	2	12 wks	Histology (IHC), biomechanical, Imaging (MRI)	Positive	B	8
Han et al[75]	PCL/nHAp/Col-wrapped tendon	Biomaterial	Rabbit	24	Hamstring (Uni)	Graft-bone interface	2	4 wks to 8 wks	Histology, biomechanical	Positive	B	6
Fleming et al[24]	PRP-ECM scaffold	Growth factor	Mini-pig	55	PET (Bi)	Ligamentization	5	15 wks	Laxity testing Histology, biomechanical	Positive	A	8
Bi et al[73]	Collagen matrix	Biomaterial	Rabbit	20	Silk (Uni)	Both	2	4 wks to 16 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	7
Song et al[121]	Augmented remnant repair	Autologous tissue	Rabbit	50	Hamstring (Uni)	Ligamentization	2	12 wks	Laxity testing, biomechanical	No difference	A	8
Shen et al[74]	Collagen matrix	Biomaterial	Rabbit	15 (30)	Silk (Bi)	Both	2	8 wks to 72 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	6
Lui et al[102]	TDSC sheet wrapped tendon	Stem cell	Rat	97	Flexor (Uni)	Both	2	2 wks to 12 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	6
Li et al[25]	TCP/PEEK anchor	Biomaterial	Pig	14	Silk (Uni)	Graft-bone interface	2	12 wks to 24 wks	Histology, biomechanical	Positive	B	7
Li et al[44]	Human fibronectin coating	Growth factor	Rat	20	PET (Uni)	Ligamentization	2	4 wks	Histology (IHC) Imaging (SEM)	Positive	C	7
Kuang et al[87]	Sr-CPC and CPC	Biomaterial	Rabbit	15 (30)	Achilles (Bi)	Graft-bone interface	2	3 wks to 24 wks	Histology (HM)	Positive	C	5
Hsu et al[76]	DBM	Bone substitute	Rabbit	10	Extensor (Uni)	Graft-bone interface	2	4 wks to 12 wks	Histology (IHC), Radiographs	Positive	C	8
Bi et al[138]	PTH (injection)	Pharmaceutical	Rat	20	Flexor (Uni)	Graft-bone interface	2	4 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	6
Zhu et al[100]	BMSC-seeded PLGA scaffold	Stem cell	Rabbit	48	Hamstring (Bi)	Graft-bone interface	3	4 wks to 12 wks	Histology, biomechanical, imaging (microCT)	Positive	B	7
Zhai et al[62]	PRP, DBP	Growth factor, Bone substitute	Rabbit	48	Hamstring (Bi)	Graft-bone interface	4	2 wks to 12 wks	Histology, biomechanical, imaging (microCT)	Positive	B	6
Weimin et al[26]	CaP cement, RBX	Biomaterial, Bone substitute	Rabbit	90	Extensor (Bi)	Graft-bone interface	3	6 wks to 24 wks	Histology, biomechanical, imaging (microCT)	Positive	B	5
Vaquette et al[72]	PolyNaSS	Biomaterial	Sheep	51	LARS (Uni)	Graft-bone interface	2	12 wks to 52 wks	Histology, biomechanical, imaging (microCT)	Positive	B	8

was seen between the two authors (intraclass correlation coefficient, ICC 0.910; 95% confidence interval 0.799 to 0.952). Most (71/92) of the studies had outcome measures ranked at 'B' (Table II).

Clinical studies. Follow-up ranged from three to 36 months (median 12). The number of patients ranged from ten to 150 (median 50). A total of 15 studies (80%) evaluated growth factors, all of which were in the form of platelet

Table II. Animal studies (n = 92) (cont.)

Author	Biological modulation	Category of intervention	Animal model	n	ACLR graft (uni/bi)	Site of action	Groups (n)	Follow-up min to max	Outcome measures	Finding	Evidence level	Quality score
Sun et al[122]	Augmented remnant repair	Autologous	Rabbit	48 (96)	Achilles (Bi)	Both	2	4 wks to 12 wks	Histology, biomechanical	Positive	B	6
Sun et al[123]	Muscle remnant (SMDSC)	Autologous tissue	Rabbit	40	Achilles (Bi)	Ligamentization	2	2 wks to 8 wks	Laxity/Gait Histology, biomechanical	Positive	A	7
Pan et al[149]	ICPC-RBX, BMP	Biomaterial, Growth factor	Rabbit	90 (180)	Extensor (Bi)	Graft-bone interface	3	6 wks to 24 wks	Histology, biomechanical, imaging (microCT)	Positive	B	6
Oka et al[139]	Simvastatin	Drug	Rabbit	42	Hamstring (Bi)	Graft-bone interface	2	2 wks to 8 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	5
Murray et al[27]	Autologous blood-ECM scaffold	Growth factor	Mini-pig	64	Patella (Uni)	Ligamentization	4	24 wks to 52 wks	Laxity Histology (IHC), biomechanical	No difference	A	7
Mifune et al[103]	ACL-derived cell sheet	Stem cell	Rat	27	Flexor (Bi)	Both	3	2 wks to 8 wks	Histology, biomechanical	Positive	B	7
Lui et al[136]	Alendronate (local)	Pharmaceutical	Rat	72	Flexor (Uni)	Graft-bone interface	3	2 wks to 6 wks	Histology, biomechanical, imaging (CT)	Positive	B	7
Lui et al[137]	Alendronate (systemic)	Pharmaceutical	Rat	84	Flexor (Uni)	Graft-bone interface	3	2 wks to 6 wks	Histology, biomechanical, imaging (CT)	Positive	B	8
Cho et al[71]	Gelatin and hyaluronic acid coating	Biomaterial	Pig	6	PET (Uni)	Graft-bone interface	2	12 wks	Histology, imaging (microCT)	Positive	C	5
Mutsuzaki et al[89]	CaP-hybridised tendon graft	Biomaterial	Goat	12	Hamstring, Flexor	Graft-bone interface	2	24 wks	Histology, biomechanical, imaging (microCT)	Positive	B	7
Mifune et al[104]	ACL-derivedCD34+ cells (injected)	Stem cell	Rat	40	Flexor (Bi)	Graft-bone interface	4	2 wks to 8 wks	Histology (IHC) biomechanical, imaging (microCT)	Positive	B	6
Matsumoto et al[119]	Augmented remnant repair	Autologous	Dog	20 (40)	Flexor (Bi)	Graft-bone interface	2	2 wks to 4 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	5
Lovric et al[78]	DBM	Bone substitute	Rat	56	Flexor (Uni)	Graft-bone interface	2	2 wks to 6 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	8
Li 2012	Bioactive glass	Biomaterial	Rabbit	30	PET (Bi)	Graft-bone interface	2	3 wks to 12 wks	Histology (IHC), biomechanical	Positive	B	6
Lee et al[28]	PRP	Growth factor	Rabbit	20	SIS (Uni)	Graft-bone interface	2	1 wks to 8 wks	Histology (IHC), biomechanical, imaging (MRI)	Negative	B	8
Kadonishi et al[79]	Enamel matrix derivative	Bone subsitute I	Rat	30	Flexor (Bi)	Graft-bone interface	2	4 wks to 12 wks	Histology, biomechanical	Positive	B	6
Dong et al[116]	BMSC-BMP2 Transfected	Gene therapy (Stem cell)	Rabbit	30	Gastroc (Bi)	Graft-bone interface	3	4 wks to 8 wks	Histology, biomechanical	Positive	B	6
Chen et al[29]	VEGF-released system	Growth factor	Rabbit	45	Patella (Bi)	Ligamentization	4	2 wks to 8 wks	Histology, biomechanical	Positive	B	6
Zhang et al[30]	BMP, RBX	Growth factor, Bone substitute	Rabbit	51	Flexor (Bi)	Graft-bone interface	3	6 wks to 12 wks	Histology, biomechanical, imaging (microCT)	Positive	B	5
Wei et al[31]	TGF-b/VEGF transfected MSC	Gene therapy (Stem cell)	Rabbit	176	Achilles (Uni)	Ligamentization	4	3 wks to 24 wks	Histology (IHC) , biomechanical	Positive	B	6
Pan et al[32]	BMP, CPC (injected) Fibrin sealant (inj.)	Growth factor, biomaterial	Rabbit	51 (102)	Extensor (Bi)	Graft-bone interface	3	2 wks to 12 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	6
Mutsuzaki et al[90]	CaP-hybridized tendon graft	Biomaterial	Goat	18	Hamstring, Flexor (Uni)	Graft-bone interface	3	52 wks	Laxity Histology, biomechanical, imaging (microCT)	Positive	A	7
Hashimoto et al[33]	rhBMP-2	Growth factor	Rabbit	40	Hamstring (Uni)	Ligamentization	2	4 wks to 8 wks	Histology, biomechanical, imaging (microCT)	Positive	B	7
Wang et al[111]	BMP-transected cells	Gene therapy	Rabbit	36	Extensor (Uni)	Graft-bone interface	2	1 wks to 12 wks	Histology (IHC), biomechanical, imaging (MRI)	Positive	B	6
Shen et al[91]	CaP ceramic	Biomaterial	Rabbit	30	Hamstring (Bi)	Graft-bone interface	2	4 wks to 12 wks	Histology (HM)	Positive	D	5
Nakase et al[34]	HGF	Growth factor	Rabbit	50	Extensor (Bi)	Graft-bone interface	2	2 wks to 12 wks	Histology, biomechanical	Positive	B	6
Kawai et al[69]	Chitin coating	Biomaterial	Rabbit	20	Polyester (Uni)	Both	2	8 wks	Histology, biomechanical	Positive	B	7
Wen et al[92]	CaP cement	Biomaterial	Rabbit	28 (56)	Extensor (Bi)	Graft-bone interface	2	6 wks to 12 wks	Histology, biomechanical, imaging (microCT)	Positive	B	6
Papatheodorou et al[145]	LIPUS	Biophysical	Rabbit	52	Extensor (Bi)	Graft-bone interface	2	3 wks	Histology (rtPCR)	Positive	C	6
Pan et al[60]	RBX	Bone substitute	Rabbit	25 (50)	Extensor (Bi)	Graft-bone interface	2	2 wks to 12 wks	Histology, biomechanical, imaging (microCT)	Positive	B	5
Lu et al[35]	BMP2- releasing interference screw	Growth factor	Sheep	14 (28)	Extensor (Bi)	Graft-bone interface	2	6 wks	Histology, biomechanical	Positive	B	6
Karaoglu et al[129]	Autogenous bone marrow aspirate, periosteum	Autologous tissue	Rabbit	36	Extensor (Bi)	Graft-bone interface	3	6 wks to 12 wks	Histology (HM) biomechanical	Positive	B	6
Fan et al[105]	MSCs	Stem cell	Pig	12	Silk (Uni)	Both	2	24 wks	Histology (IHC), biomechanical, imaging (microCT)	Positive	B	6
Sasaki et al[36]	G-CSF	Growth factor	Dog	28 (56)	Flexor (Bi)	Graft-bone interface	2	2 wks to 4 wks	Histology, biomechanical, imaging (microCT)	Positive	B	6
Gulotta et al[80]	Mg-based bone adhesive	Bone substitute	Rabbits	35 (70)	Hamstring (Bi)	Graft-bone interface	2	3 wks to 6 wks	Histology, biomechanical, imaging (microCT)	Positive	B	7
Fan et al[106]	MSC	Stem cell	Rabbit	48	Silk (Uni)	Both	2	8 wks to 24 wks	Histology, biomechanical imaging (microCT)	Positive	B	7
Yeh et al[141]	Hyperbaric oxygen	Environmental	Rabbit	40	Hamstring (Uni)	Both	2	6 wks to 18 wks	Histology, biomechanical	Positive	B	8
Walsh et al[144]	LIPUS	Biophysical	Sheep	89	Extensor (Uni)	Graft-bone interface	2	3 wks to 26 wks	Histology, biomechanical	Positive	B	8
Soon et al[107]	MSC-coated allografts	Stem cell	Rabbit	36 (72)	Achilles (Bi)	Graft-bone interface	2	2 wks to 8 wks	Histology, biomechanical	Positive	B	7

concentrates, such as PRP; the remainder studied platelet rich fibrin matrix, autologous serum, platelet leucocyte gel and autologous platelet concentrate. All interventions were administered at the time of surgery except four studies which included post-operative interventions. These included one study evaluating postoperative extracorporeal shockwave therapy (ESWT)[147] and three studies evaluating post-operative joint injections of growth factors in the form

Table II. Animal studies (n = 92) (cont.)

Author	Biological modulation	Category of intervention	Animal model	n	ACLR graft (uni/bi)	Site of action	Groups (n)	Follow-up min to max	Outcome measures	Finding	Evidence level	Quality score
Ma et al[37]	rhBMP-2	Growth factor	Rabbit	60 (120)	Hamstring (Bi)	Graft-bone interface	3	2 wks to 8 wks	Histology (HM), biomechanical	Positive	B	6
Li et al[117]	MSC, PDGF-B transfected MSC	Gene therapy (Stem cell)	Rabbit	36 (72)	Achilles (Bi)	Ligamentization	2	3 wks to 12 wks	Histology,	Positive	E	4
Huangfu et al[93]	TCP	Biomaterial	Dog	48 (96)	Flexor (Bi)	Graft-bone interface	2	2 wks to 12 wks	Histology, biomechanical	Positive	B	6
Yoshikawa et al[38]	VEGF	Growth factor	Sheep	18	Hamstring (Uni)	Ligamentization	2	12 wks	Laxity histology biomechanical	Negative	A	8
Yamazaki et al[39]	TGFb1	Growth factor	Dog	21	Flexor (Uni)	Graft-bone interface	3	3 wks	Histology, biomechanical	Positive	B	8
Wang et al[146]	Shock wave therapy	Biophysical	Rabbit	36	Extensor (Bi)	Graft-bone interface	2	1 wk to 24 wks	Histology (HM), biomechanical	Positive	B	8
Demirag et al[135]	α_2-macroglobulin (MMP inhibitor)	Drug	Rabbit	28 (56)	Hamstring (Bi)	Graft-bone interface	2	2 wks to 5 wks	Histology, biomechanical	Positive	B	7
Yasuda et al[40]	EGF and TGF β	Growth factor	Dog	20 (40)	Patella (Bi)	Ligamentization	4	12 wks	Histology, biomechanical	Positive	B	7
Weiler et al[41]	PDGF-coated sutures	Growth factor	Sheep	48	Flexor (Uni)	Ligamentization	2	3 wks to 24 wks	Laxity histology (IHC), biomechanical	No difference	A	6
Tien et al[94]	CaP cement	Biomaterial	Rabbit	22 (44)	Hamstring (Bi)	Graft-bone interface	2	1 wk to 24 wks	Histology, biomechanical	Positive	B	6
Mutsuzaki et al[95]	CaP-hybridized tendon	Biomaterial	Rabbit	50 (100)	Flexor (Bi)	Graft-bone interface	2	1 wk to 3 wks	Histology	Positive	D	4
Mihelic et al[43]	BMP-7	Growth factor	Sheep	30	Peroneal (Uni)	Graft-bone interface	2	3 wks to 6 wks	Histology (HM), biomechanical	Positive	B	7
Lim et al[108]	MSC	Stem cell	Rabbit	48 (96)	Hamstring (Bi)	Graft-bone interface	2	2 wks to 8 wks	Histology (IHC), biomechanical	Positive	B	5
Martinek et al[110]	BMP-2 genetically altered ST graft	Gene therapy	Rabbit	48	Hamstring (Bi)	Graft-bone interface	3	2 wks to 8 wks	Histology, biomechanical	Positive	B	6
Chen et al[130]	Autologous periosteum	Autologous tissue	Rabbit	36 (72)	Hamstring (Bi)	Graft-bone interface	2	4 wks to 12 wks	Histology, biomechanical	Positive	B	4
Anderson et al[42]	Bone-derived extract	Growth factor	Rabbit	70 (140)	Hamstring (Bi)	Graft-bone interface	2	2 wks to 8 wks	Histology, biomechanical, imaging (MRI)	Positive	B	7

of PRP[48,56] or autologous serum.[51] The majority of studies explicitly targeted both the graft and the tunnels with intraoperative application of a biological intervention (n = 12), whereas six studies specifically targeted the tunnels.

Positive results were reported by nine of 20 studies; including seven studies of platelet concentrates, one study of calcium phosphate hybridized tendon, and one of ESWT. Two clinical studies, using remnant repair and bone substitutes, had mixed findings, reporting positive radiological outcomes, but no clinical differences. The remaining nine studies (eight studies of platelet concentrates and one study of bone marrow derived mesenchymal stem cells) reported no effect of the intervention.

Study quality. A total of 15 studies were randomized controlled trials and five studies were non-randomized studies (three case control studies and two prospective cohort studies). The methodological quality scores for clinical studies were seen in the following frequency: one study had low levels of concern for methodological quality, there were moderate concerns in 17 (85%) and serious concerns in two studies (10%). Of the randomized studies, 14/15 had moderate risk of bias either due to lack of clarity of blinding status, lack of clarity of the randomization process, or inadequate blinding of the operative surgeon. All observational studies had moderate risk of bias due to inadequate adjustment for potential confounders. Studies given a serious risk of bias also had potential bias in the selection of participants into the study. Acceptable inter-rater reliability was seen between the two authors (ICC 0.734; 95% confidence interval 0.372 to 0.904).

Out of the 14 studies evaluating PRP, none met the minimum reporting standards, with a median score of 12/23 (range 9 to 18). One study evaluated MSCs which had a score of 19/25 against the minimum reporting standards.

Discussion

A total of 24 different technologies were identified in the basic science literature, falling into seven broad categories. Of these 24, six have progressed to clinical studies, the majority of which (15/20) concern the use of PRP (Table IV).

Growth factors. Most studies (39, of which 15 were clinical studies) concern growth factors, administered either as individual factors or as platelet concentrates.[21-59] Growth factors studied in animals included bone morphogenetic growth proteins (BMPs)[30,35,37,60,61] basic fibroblast growth factor (bFGF),[12] epidermal growth factor (EGF),[40] granulocyte colony-stimulating factor (g-CSF),[36] hepatocyte growth factor (HGF),[34] transforming growth factor-β (TGF-β),[39] and vascular endothelial growth factor (VEGF).[29,31,38] Platelet concentrates, which are sources of bioactive molecules and multiple growth factors such as platelet-derived growth factor (PDGF), TGF-β and VEGF, include PRP,[21,24,28,29,31,38,62] fibrin clot[23] and autologous conditioned serum.[51] Growth factors are thought to enhance the healing process[63] and can be used to target graft healing; both on the graft-tunnel interface (n = 13 studies), and to promote intra-articular ligamentization (n = 11 studies).

In total 21 of the 24 animal studies that evaluated growth factors showed positive findings, but two animal studies showed no effect of growth factors, which included PDGF-coated sutures in sheep[41] and autologous blood

Table III. Clinical studies (n = 20)

Author	Biological modulation	Type	Site of action (and time)	Patients (n)	Surgery (graft)	Groups (n)	Follow-up min to max	Outcome measures	Finding	Study design	Reporting standards reached	Risk of bias
Del Torto et al[45]	PRFM	Growth factor	Tunnel (intraoperative)	28	Hamstring	2	1 y to 2 yrs	Laxity (rolimeter) Imaging (MRI) Functional Score (IKDC)	No difference	Non-RCT (prospective)	No (13/23)	Moderate
Wang et al[147]	ESWT	Biophysical	Tunnel (single dose after wound closure)	53	Hamstring	2	6 mths to 2 yrs	Laxity (arthrometer) Imaging (MRI) Functional Score (Lysholme, IKDC)	Positive	RCT	N/A	Moderate
Valentí Azcárate et al[46]	PRP	Growth factor	Tunnel and graft (intraoperative)	150	Patella	3	3 mths to 12 mths	Imaging (MRI) Functional Score (IKDC)	No difference	RCT	No (17/23)	Moderate
Silva et al[109]	BMSC	Autologous MSCs	Tunnel and graft (intraoperative)	43	Hamstring	2	3 mths	Imaging (MRI)	No difference	RCT	No (19/25)	Moderate
Matsumoto et al[127]	Augmented remnant repair	Autologous tissue	Tunnel and graft (intraoperative)	10	Hamstring	2	1 y to 2 yrs	Laxity (arthrometer) Imaging (CT) Functional Score (Lysholme, IKDC)	Positive radiologically (no difference clinically)	RCT	N/A	Moderate
Vadalà et al[47]	PRP	Growth factor	Tunnel and graft (intraoperative)	40	Hamstring	2	10 mths to 16 mths	Laxity (arthrometer) Imaging (CT) Functional Score (Lysholme, Tegner, IKDC)	No difference	RCT	No (9/23)	Moderate
Seijas et al[48]	PRP	Growth factor	Tunnel and graft (postoperative joint injection)	98	Patella	2	4 mths to 12 mths	Imaging (MRI)	Positive	RCT	No (10/23)	Moderate
Rupreht et al[49]	PRP	Growth factor	Tunnel and graft (intraoperative)	50	Hamstring	2	6 mths	Imaging (MRI)	Positive	RCT	No (12/23)	Moderate
Mirzatolooei et al[50]	PRP	Growth factor	Tunnel and graft (Intraoperative)	50	Hamstring	2	3 mths	Laxity (arthrometer) Imaging (CT)	No difference	RCT	No (14/23)	Moderate
Iorio et al[85]	Nanohydroxyapatite bone substitute	Biomaterial	Tunnel and graft (intraoperative)	40	Hamstring	2	30 days to 180 days	Laxity (arthrometer) Imaging (MRI) Functional Score (Lysholme, Tegner, IKDC)	Positive radiologically (no difference clinically)	RCT	N/A	Moderate
Mutsuzaki et al[89]	CaP-hybridized tendon graft	Biomaterial	Tunnel and graft (intraoperative)	64	Hamstring	2	1 y to 2 yrs	Laxity (arthrometer) Imaging (MRI and CT) Arthroscopy Functional Score (Lysholme, Tegner, IKDC)	Positive	RCT	N/A	Moderate
Darabos et al[51]	Autologous serum	Growth factor	Tunnel and graft (4x postoperative joint injections)	62	Hamstring	2	12 mths	IL-1β synovial fluid conc. Imaging (CT) Functional Score (WOMAC, IKDC)	Positive	RCT	N/A	Low
Vogrin et al[52]	PLG	Growth factor	Tunnel and graft (intraoperative)	50	Hamstring	2	3 mths to 6 mths	Laxity (arthrometer)	Positive	RCT	No (12/23)	Moderate
Sánchez et al[53]	PRP	Growth factor	Tunnel and graft (intraoperative)	37	Hamstring	2	6 mths to 24 mths	Arthroscopy Histology (LTMI)	Positive	Non-RCT (case con)	No (11/23)	Moderate
Radice et al[54]	PRP	Growth factor	Tunnel and graft (Intraoperative)	50	Hamstring,BTPB	2	3 mths to 12 mths	Imaging (MRI)	Positive	Non-RCT (case con)	No (9/13)	Serious
Figueroa et al[55]	APC	Growth factor	Tunnel and graft (intraoperative)	50	Hamstring	2	6 mths	Imaging (MRI)	No difference	Non-RCT (case con)	No (10)	Moderate
Silva et al[56]	PRP	Growth factor	Tunnel (intraoperative application and postoperative injections)	40	Hamstring	4	3 mths	Imaging (MRI)	No difference	Non-RCT (prospective)	No (11/23)	Serious
Nin et al[57]	PRP	Growth factor	Tunnel and graft (intraoperative)	100	Patella	2	18 mths to 36 mths	Laxity (arthrometer) Imaging (MRI) Functional Score (IKDC)	No difference	RCT	No (18/23)	Moderate
Orrego et al[58]	APC	Growth factor	Tunnel and graft (intraoperative)	108	Hamstring	4	3 mths to 6 mths	Imaging (MRI)	No difference	RCT	No (12/23)	Moderate
Ventura et al[59]	PRP	Growth factor	Tunnel (intraoperative)	20	Hamstring	2	6 mths	Laxity (arthrometer) Imaging (CT) Functional Score (Tegner, KOOS)	Positive	RCT	No (10/23)	Moderate

ACL, anterior cruciate ligament; ACLF. anterior cruciate ligament fibroblasts (ACLFs); AT, achilles tendon; Allo, allograft; APC, autologous platelet concentrate; ADSCs, adipose-derived stem cells; BMSCs, bone marrow derived mesenchymal stem cells; BMP-2, bone morphogenetic protein 2; bFGF, basic fibroblast growth factor; BMSC, bone marrow mesenchymal stem cells; Bio-scaffold, biologically active scaffold; Bi, bilateral reconstruction; BPTB, bone–patellar tendon–bone autograft; CAPP, ceramide-activated protein phosphatase; CaP, calcium phosphate; Case con, case control; DBM, demineralised bone matrix; DCB, demineralised cortical bone; DPB, deproteinized bone; DBP, demineralized bone protein; ESWT, extracorporeal shockwave therapy; EXT, extensor tendon; Extra, extra-articular; ECM, extracellular matrix; EGF, epidermal growth factor; Flex, flexor tendon; G-CSF, Granulocyte colony-stimulating factor; Gastroc, gastrocnemius; GF, growth factor; HT, hamstring; HM, Histomorphometric analysis.; hAT-MSCs, human adipose tissue; hiPSC-MSCs, induced pluripotent stemcells generated from human foreskin fibroblasts; HAP, hydroxyapatite; HGF, hepatocyte growth factor; hUCB-MSCs, human umbilical cord blood derived mesenchymal stem cells; IFS, injected fibrin sealant; ICPC, injected calcium phosphate cement; IHC, immunohistochemistry; LARS, Ligament Advanced Reinforcement System; LIPUS, low-intensity pulsed ultrasound; LHA, low crystallinity hydroxyapatite; LTMI, ligament tissue maturity index; Mg, magnesium; MMP, matrix metalloproteinases; MSCs, mesenchymal stem cells; OPG, osteoprotegerin; PLGA, poly(d,l-lactide-*co*-glycolide); PCL/nHAp/Col, polycaprolactone/nanohydroxyapatite/collagen; PDGF, platelet-derived growth factor; PRFM, platelet-rich fibrin matrix; PLA, polylactide; PLG, Platelet-leucocyte gel; PCL, polycaprolactone; PET, polyethylene terephthalate; PRP, platelet-rich plasma; PolyNaSS , polystyrene sodium sulfonate; PT, patella tendon; PRFM, platelet rich fibrin matrix; PDGF, platelet-derived growth factor; PEEK, polyether ether ketone; PTH, parathyroid hormone; Peroneal, peroneus longus; PP, Partial patellectomy; PLG, platelet leucocyte gel; rhBMP-2, recombinant human bone morphogenetic protein-2; RBX, recombined bone xenograft; rtPCR, reverse transcription polymerase chain reaction; RCT, randomised controlled trial; ST, semitendinosus; siRNA, small interfering RNA; Sr-CPC, strontium-enriched calcium phosphate cement; sFLT1: soluble fms-like tyrosine kinase-1; SMDSC, skeletal muscle derived stem cells; Scaff, scaffold; ST, semitendinosis; STG, semitendinosus and gracilis grafts; Sr-CPC, strontium-enriched calcium phosphate cement; SIS, small intestine submucosa; TGF, transforming growth factor; TDSCs, Tendon-derived stem cells; Tib, tibialis anterior; TCP, tricalcium phosphate; TGF, transforming growth factor; TCP, tricalcium phosphate; Untra, untracduced; Uni, unilateral reconstruction; Xeno, xenogenic; VEGF, vascular endothelial growth factor

applied to an extracellular matrix scaffold in pigs.[27] The animal study where a negative effect was observed was a study where 18 sheep received semitendinosus grafts soaked in VEGF; while this promoted angiogenesis, it also led to reduced biomechanical strength and knee stability at 12 weeks.[38] Conversely in a separate study, the use of a sodium hyaluronate VEGF-releasing system increased ligamentization of patellar tendons in rabbits.[29]

In clinical trials, the most extensively studied biological intervention is PRP, which can be applied directly to the graft site (graft substance and graft-tunnel interface), or indirectly through intra-articular injection. A systematic review in 2015[64] suggested that PRP may promote ligamentization, but there is less evidence to suggest it enhances osseointegration. The largest randomized controlled trial (RCT) to date evaluated 150 patients with a

Table IV. Categories of biological augmentation of anterior cruciate ligament reconstruction (ACLR)

Category	Intervention	Comparative clinical trials (n)	Clinical findings
Growth factors	Individual growth factors eg. bFGF, BMPs-2, VEGF	0	N/A
	Blood/platelet concentrates eg. PRP, Fibrin clot	15	Mixed
Biomaterials	Biological fixation eg. Magnesium interference screws, PLA bolts	0	N/A
	Biological coatings e.g. Bio-active glass, Chitin, Collagen matrix, Gelatin and hyaluronic acid, Fibronectin, PLGA, PEEK, PLA, PolyNaSS	0	N/A
	Biosynthetic bone substitutes e.g. Demineralised Bone Matrix, Recombined bone xenograft	1	No difference
	Osteoconductive materials e.g. Calcium phosphate (CaP) and Strontium-enriched CaP, CaP-hybridised tendon	1	Positive
Stem cells	ACL-derived vascular stem cells (CD34+)	0	N/A
	Adipose-derived stem cells	1	
	Adult non-cultivated bone marrow stem cells	0	Positive
	Bone marrow derived mesenchymal stem cells (BMSC)	0	N/A
	Induced pluripotent stem cells	0	N/A
	Tendon-derived stem cells	0	N/A
	Umbilical cord stem cells	0	
Autologous tissue	Periosteum	0	N/A
	Remnant tissue eg. ACL, muscle	1	Positive
Gene therapy	Based on stem cells eg. ACL CD34+ -BMP2 transfected, BMSC - bFGF/BMP2 transfected	0	N/A
	Based on graft tissue eg. BMP-2 genetically altered semitendinosis graft	0	N/A
Pharmaceuticals	Bisphosphonates eg. Alendronate	0	N/A
	Matrix metalloproteinases inhibitors eg. Alpha- 2-macroglobulin	0	N/A
	Parathyroid hormone	0	N/A
	Simvastatin	0	N/A
Biophysical and environmental	Extracorporeal shockwave therapy	1	Positive
	Low-intensity pulsed ultrasound, LIPUS	0	N/A
	Hyperbaric oxygen	0	N/A

bFGF, basic fibroblast growth factor;BMP-2, bone morphogenetic protein 2; VEGF, vascular endothelial growth factor; PRP, platelet-rich plasma; PLA, polylactide; PLGA, poly(d,l-lactide-*co*-glycolide); PEEK, polyether ether ketone; PolyNaSS , polystyrene sodium sulfonate; N/A, not applicable

control group and two intervention groups with different concentrations of PRP and found no difference in radiological graft healing, or on clinical examination.[46] Schippinger et al[65] showed PRP harvested from patients receiving non-steroidal anti-inflammatories had inferior platelet function and bioactivity compared with controls.

Biomaterials. This group encompasses four separate technologies: biological fixation methods; biological coatings; biosynthetic bone substitutes; and osteoconductive materials. Two studies have demonstrated that magnesium-based interference screws can accelerate graft mineralization, promote bone formation in the periscrew region, and enhance osseointegration of extensor tendon in rabbit models of ACLR.[66,67] The use biodegradable polylactide (PLA) bolts as the bone anchor and poly(d,l-lactide-co-glycolide) (PLGA) nanofibrous membrane at the graft-tunnel interface has been shown to enhance bone ingrowth and reduce peritunnel bone loss in rabbits.[68] Studies have shown graft-tunnel interface healing of artificial grafts can be enhanced using coatings such as chitin,[69] bioglass,[70] gelatin and hyaluronic acid,[71] polystyrene sodium sulfonate[72] and collagen matrix.[73,74] Similar coatings have been used with tendon autograft; for instance the use of a hydroxyapatite-doped polycaprolactone nanofiber membrane wrapped around autograft hamstring tendon in rabbits enhanced tissue integration and mechanical strength.[75] Biosynthetic bone substitutes, such as demineralized bone matrix and recombinant bone xenograft, are promising biomaterials for enhancing graft-tunnel interface healing because they are both osteoinductive and osteoconductive, and they have been evaluated in eight animal studies, all with positive findings.[26,30,62,76-80] Demineralized bone matrix (DBM) is manufactured by acid extraction of the mineral component of bone, which leaves collagen scaffold containing growth factors such as BMPs, and has shown the ability to enhance tendon-bone healing in animal models of rotator cuff pathology.[81-84] When applied to the tendon-bone interface in ACLR, DBM can augment tendon-bone healing in rabbit[76] and rat[78] models of ACLR. In 2013, Iorio et al[85] reported the only clinical study to evaluate a bone substitute in graft healing when 40 patients were randomized to conventional ACLR with single-bundle hamstring reconstruction or nanohydroxyapatite bone-based graft, as well as ACLR. Whilst this was a short-term follow up, the intervention group showed superior radiological assessment of graft healing, at long-term follow up there was no difference between the groups in

terms of radiological and clinical outcome measures. Calcium phosphate (CaP) is a resorbable and osteoconductive biomaterial that has shown positive results in 12 animal studies which evaluated graft healing.[25,32,86-95] There is evidence that CaP materials and cements can enhance tendon-bone healing in rabbit[32,91,92,94] and dog[93] animal models, an effect which is enhanced with incorporation of strontium.[87,88] Mutsuzaki et al[86] investigated a CaP-hybridized flexor tendon in a goat model (n = 10) and after six months, animals treated with the CaP-hybridized tendon showed superior knee stability and increased osseointegration at the graft-tunnel interface. A randomized controlled trial[89] was carried out in humans involving 64 patients, who were randomized to receive conventional hamstring ACLR or CaP-hybridized graft reconstruction where tendon graft was hybridized intraoperatively with CaP at both ends of the graft. When compared with the control group, the hybridized-group showed reduced tunnel enlargement at one year and superior anterior knee stability and Lysholm scores at two-year follow-up.

Stem cells. Multiple stem cell types have been shown to enhance graft-tunnel interface healing in animal models. Cells can be isolated at the time of surgery[109] using bone marrow aspirate, or cells can be culture-expanded prior to the surgical procedure. Cells that have been culture expanded include adipose-derived stem cells (ADSCs),[96-98] bone marrow derived stem cells (BMSCs),[99,100] induced pluripotent stem cells (iPSCs),[97] umbilical cord-derived mesenchymal stem cells,[101] tendon-derived stem cells[102] and CD34+ ACL-derived stem cells.[103,104] Stem cells have been seeded on scaffold,[96,99,100,105-107] in the form of sheets[102,103] or applied locally to grafts.[108] Li et al[99] found that a BMSC-seeded silk scaffold implanted in rabbits led to enhanced osseointegration at 24 weeks confirmed by biomechanical testing, micro-CT and histological assessment. Kosaka et al[98] found that a coating semitendinosus graft with ADSCs mixed in a fibrin glue carrier in a rabbit model led to higher ultimate failure load and enhanced histological remodelling by 12 weeks. In a rat model, Mifune et al[103] found that wrapping a tendon graft in an ACL-derived CD34+ cell sheet demonstrated increased biomechanical strength. The CD34 antigen is expressed on haematopoietic stem and progenitor cells. Immunohistochemistry confirmed that cells derived from the cell sheets had integrated within the tendon graft and the graft-tunnel interface.[103] Intracapsular administration of ACL-derived CD34+ cells in a rat model led to recruitment of the transplanted cells into the perigraft site, with enhanced angiogenesis, osteogenesis, and biomechanical strength.[104]

The only clinical study evaluating stem cells in tendon-bone healing was a RCT of 44 patients where adult non-cultivated bone marrow stem cells were obtained intraoperatively.[109] Bone marrow harvested from the iliac crest was centrifuged and 3 ml of marrow stem cell concentrate was obtained, which was applied to the femoral side of the graft and the femoral osseous tunnel. MRI assessment of tendon-bone healing found no difference between intervention and control groups at three months.

Gene therapy. Martinek et al[110] reported the first study to evaluate gene therapy on the graft-tunnel interface after ACLR. A rabbit model of ACLR injury was used, and rabbits either received an untreated semitendinosus tendon, or semitendinosus tendon infected *in vitro* with adenovirus-BMP-2 (AdBMP-2). Infection was accomplished by culturing the semitendinosus tendon with the adenovirus prior to the operation. The number of infected cells was stable during the eight-week study. Improved integration of semitendinosus tendon grafts within the bone tunnels was observed with associated improvements in biomechanical strength. In a rabbit extensor tendon model, Wang et al[111] applied BMP-2 gene-transfected normal rat kidney cells at the tendon-bone interface. After 12 weeks the study group had superior biomechanical strength and histological assessment showed greater integration between tendon and bone compared with controls with higher expressions of BMP-2 and VEGR on immunohistochemistry. Besides the previous two studies, all other gene therapy approaches to enhance graft healing has been based on stem cells.[31,112-117] By transfecting stem cells with growth factors such as BMP2, PDGF-B and TGF-β, a stable continuous concentration of these agents would be delivered to the graft healing site.[118] Unlike local application of growth factors intraoperatively, genetic modification of stem cells offers the benefits of a strong and sustained effect of growth factors. Chen et al[114] found that implantation of genetically modified MSCs with bFGF and BMP2 at the graft-tunnel interface in rabbit model of ACLR led to improved cellularity, enhanced new bone formation, and conferred superior mechanical properties. Furthermore, the co-application of these two genes was more powerful and efficient than either single gene therapy.

Autologous tissue. When the ACL ruptures, CD34+ vascular stem cells released locally are thought to contribute to healing at the site of rupture.[119] An advantage of using autologous tissue over cultured stem cells is the ability to deliver a source of regenerative cells without the risk of rejection and malignancy. Attachment of the ACL remnant to the graft is intended to promote ligamentization. Six animal studies have been carried out evaluating the effect of remnant repair on graft healing,[120-125] with positive findings in four studies and no difference in two. Song et al[125] found no difference in biomechanical strength, revascularization status, and proprioceptive recovery potential between 30 rabbits who underwent remnant-preserving ACLR and 30 rabbits who underwent conventional ACLR. Conversely, in a study of 42 sheep, Takahashi et al[124] found that remnant-preserving ACLR enhanced revascularization and regeneration of proprioceptive organs compared with conventional ACLR,

leading to reduced anteroposterior laxity on clinical examination. However, there was no improvement in biomechanical strength. Matsumoto et al[126] found that autologous ruptured ACL tissue sutured to the tibial side of the graft enhanced maturation of bone-tendon integration in a canine model of ACLR at four weeks and compared with an untreated-control group, and was associated with smaller tibial bone tunnels and greater ultimate tensile strength.

The effect of remnant preservation was examined in humans by Matsumoto et al[127] in 2014. In this study, five patients underwent conventional double-bundle ACLR and five patients underwent ACLR with suturing of the ruptured tissue to hamstring graft. After two years, CT scans demonstrated smaller tunnel volumes in the remnant-preserving ACLR, but no difference was detected clinically in terms of knee laxity and Lysholm score. This study was limited by follow-up occurring at different timepoints and longer-term studies are required to investigate the effects of remnant-preserving ACLR better.

The periosteum is also a source of regenerative cells capable of promoting osteogenesis and chondrogenesis and in theory the use of periosteal cell graft healing may recreate a fibrocartilaginous direct-type enthesis.[128,129] Chen et al[130] first investigated the benefit of periosteum in 36 rabbits who underwent bilateral ACLR using extensor tendons bilaterally, with one side wrapped in periosteum. After 12 weeks histologically, there was enhanced tendon-bone integration with the periosteum-enveloped tendon and associated improvements biomechanically with higher maximal pull-out forces. Improved osseointegration has also been observed when synthetic grafts are wrapped in periosteum in a goat animal model.[131] One group has reported clinical studies, albeit with no control group, which show minimal tunnel widening when hamstring tendons were enveloped in periosteum.[132-134]

Pharmaceuticals. Four different pharmaceuticals have been studied in animal models to modulate the inflammatory response present after ACL injury and augment graft healing. Demirag et al[135] investigated the effect of metal matrix metalloproteinase (MMP) inhibitors on graft healing in 28 rabbits undergoing bilateral ACLR by injecting the MMP-inhibitor alpha-2-macroglobulin into one knee. After five weeks knees that received MMP blockade showed a more mature graft-tunnel interface, reduced MMPs in the synovial fluid and better biomechanical strength.[135] In 2013 two studies[136,137] investigated the effect of bisphosphonates of osseointegration after ACLR in a rat model of ACLR. Local[136] or systemic[137] administration of alendronate resulted in improved bone tunnel mineralization, reduced peritunnel bone loss and enhanced graft-tunnel integration after six weeks. A study comparing rats receiving daily subcutaneous parathyroid hormone showed enhanced thickness and microarchitecture of trabecular bone on CT compared with rats that received saline injections.[138] Simvastatin has been shown to promote bone formation.[139] In 2016, Zhang et al[140] investigated the effect of statins on osseointegration in 36 rabbits undergoing ACLR with a PET artificial ligament. Rabbits in which the graft was coated with collagen-containing low-dose simvastatin polycaprolactone microspheres had superior biomechanical strength and better histological findings at eight weeks, with immunohistochemistry demonstrating enhanced angiogenesis and osteogenesis.

Biophysical and environmental. Yeh et al[141] studied the use of hyperbaric oxygen (HBO) on graft neovascularization in a rabbit model (n = 40). Animals receiving HBO treatment showed superior neovascularization, osseointegration and biomechanical properties. Low intensity pulsed ultrasound (LIPUS) has been used in two animal studies with positive results.[142,143] A study of 89 sheep investigating the effect of daily LIPUS found that the LIPUS group demonstrated better histological osseointegration and increased mechanical strength 26 weeks after surgery.[142] A subsequent study in rabbits found that LIPUS-treated animals had improved ligamentization, which was attributed to upregulation of genes such as TGF-1.[143] Wang et al[144] reported that ESWT applied to the tibial tunnel immediately after ACLR in rabbits improved healing at the graft-tunnel interface both histologically and biomechanically at eight and 24 weeks.

A clinical RCT of 53 patients undergoing single-bundle ACLR with hamstring autograft found that patients randomized to receive ESWT on the day of surgery had significantly superior Lysholm scores and reduced tibial peritunnel bone loss than the control group at two years.[145]

Clinical translation and future research. This systematic review demonstrates the large amount of preclinical research in the biological augmentation of ACLR. Nevertheless, the impact of the research is limited by the fact the majority of studies have used small animal models (n = 67, 60%), and only 10% of studies have used quantitative clinically useful outcome measures (as determined by rank A in Table II). We believe future research should use larger animal models as this lends itself to clinically useful outcomes such as joint laxity and gait analysis. The median follow-up of animal research is 12 weeks, but this is likely to be inadequate to assess ligamentization and the development of osteoarthritis. It is thought the poor capacity of the ACL to heal is due to the fact that the synovial fluid environment can disrupt the healing scaffold,[146] and future research should show that interventions are capable of remaining at the graft interface by using tracking methods such as quantum dot labelling.[147] In terms of clinical studies, 75% have evaluated PRP but currently there is a lack of evidence to support its efficacy and most clinical studies fall short of the minimum reporting standards. Biomaterials are the most investigated category of biological therapy in animal studies, but represent just 10% (n = 2) of clinical studies. Future clinical studies investigating biomaterials are

required, especially materials currently in use in clinical practice such as DBM, which has shown efficacy in animal studies.[148] To date, one stem cell study[109] has been performed in the context of ACLR, but with the current enthusiasm for MSC-based therapies in the treatment of musculoskeletal conditions, and the development of methods to harvest and intraoperatively deliver adult non-cultivated bone marrow stem cells, this is set to increase. There is no current clinical evidence to support the use of stem cells in ACLR, but it is possible that techniques where stem cells are delivered in higher concentrations will lead to a clinically detectable effect. Other autologous sources of stem cells requiring further evaluation in clinical studies include enhancement of the graft with the native graft remnant[119-122] and periosteum,[130,131] which have been shown to be effective in animal studies. Future research is needed to delineate the exact mechanisms of the interventions and determine whether a combination of different therapies is of benefit in the clinical setting. Despite promising results in animal studies, gene therapy remains experimental and risks such as mutagenesis and malignancy need to be addressed before clinical studies are performed.

This study has highlighted the diversity of biological therapies and of methods of delivery. To facilitate robust critical appraisal future clinical studies should comply by the published minimum standards of reporting. Relatively few clinical studies (9/20) have evaluated clinical outcome measures (knee laxity, functional scores) with imaging of the graft-bone interfaces (CT or MRI). In order to evaluate the clinical efficacy of biological studies, clinical studies should ideally include 3D imaging of the bone tunnels and the graft as well as clinical assessment of joint function, including examination of laxity and clinical functional scores.

In conclusion, this systematic review has summarized a significant body of literature on biological modulation of ACLR across human and animal models in clinical and experimental settings. As a result, there is a significant amount of heterogeneity amongst the subgroups and treatment modalities, which makes meta-analysis inappropriate. Nevertheless, this study adds important knowledge to the field. When compared with the most recent systematic review (n = 60 studies),[20] this study has reported a further 62 studies. Unlike the previous review, this study focuses exclusively on ACLR and has excluded bioenhanced repair. In addition, for the first time this study has specifically reported the target site and timing of biological therapies and has critiqued clinical studies against the minimum reporting standards.

Further clinical studies that meet the minimum standards of reporting are required to determine whether current and emerging biological strategies will provide tangible benefits in patients undergoing ACLR.

Take home message:

-The outcome of ACL reconstruction depends on a biological healing response, called "Graft Healing".

-We have systematically reviewed biological augmentation of ACL reconstruction.

-We have identified seven categories of biological intervention.

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References

1. **Prodromos CC, Han Y, Rogowski J, Joyce B, Shi K.** A meta-analysis of the incidence of anterior cruciate ligament tears as a function of gender, sport, and a knee injury–reduction regimen. *Arthroscopy* 2007;23:1320–1325.
2. **Linko E, Harilainen A, Malmivaara A, Seitsalo S.** Surgical versus conservative interventions for anterior cruciate ligament ruptures in adults. *Cochrane Database Syst Rev* 2005;2:CD001356.
3. **Wasserstein D, Sheth U, Cabrera A, Spindler KP.** A systematic review of failed anterior cruciate ligament reconstruction with autograft compared with allograft in young patients. *Sports Health* 2015;7:207–216.
4. **Grassi A, Nitri M, Moulton SG, et al.** Does the type of graft affect the outcome of revision anterior cruciate ligament reconstruction? a meta-analysis of 32 studies. *Bone Joint J* 2017;99-B:714–723.
5. **Śmigielski R, Zdanowicz U, Drwięga M, Ciszek B, Williams A.** The anatomy of the anterior cruciate ligament and its relevance to the technique of reconstruction. *Bone Joint J* 2016;98-B:1020–1026.
6. **Boddu C, Arif SK, Hussain MM, et al.** Prevention of graft-tunnel mismatch during anatomical anterior cruciate ligament reconstruction using a bone-patellar tendon-bone graft. *Bone Joint J* 2015;97-B:324–328.
7. **Shah VM, Andrews JR, Fleisig GS, McMichael CS, Lemak LJ.** Return to play after anterior cruciate ligament reconstruction in National Football League athletes. *Am J Sports Med* 2010;38:2233–2239.
8. **Paterno MV, Rauh MJ, Schmitt LC, Ford KR, Hewett TE.** Incidence of second ACL injuries 2 years after primary ACL reconstruction and return to sport. *Am J Sports Med* 2014;42:1567–1573.
9. **Ahmed I, Salmon L, Roe J, Pinczewski L.** The long-term clinical and radiological outcomes in patients who suffer recurrent injuries to the anterior cruciate ligament after reconstruction. *Bone Joint J* 2017;99-B:337–343.
10. **Kuršumović K, Charalambous C.** Graft salvage following infected anterior cruciate ligament reconstruction: a systematic review and meta-analysis. *Bone Joint J* 2016;98-B:608–615.
11. **von Porat A, Roos EM, Roos H.** High prevalence of osteoarthritis 14 years after an anterior cruciate ligament tear in male soccer players: a study of radiographic and patient relevant outcomes. *Ann Rheum Dis* 2004;63:269–273.
12. **Gulotta LV, Rodeo SA.** Biology of autograft and allograft healing in anterior cruciate ligament reconstruction. *Clin Sports Med* 2007;26:509–524.
13. **Benjamin M, Toumi H, Ralphs J, et al.** Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. *J Anat* 2006;208:471–490.
14. **Lazarides AL, Eward WC, Green K, et al.** Histological Evaluation of Tendon-Bone Healing of an Anterior Cruciate Ligament Hamstring Graft in a 14-Year-Old Boy. *Am J Sports Med* 2015;43:1935–1940.
15. **LaPrade RF, Dragoo JL, Koh JL, et al.** AAOS Research Symposium Updates and Consensus: Biologic Treatment of Orthopaedic Injuries. *J Am Acad Orthop Surg* 2016;24:62–78.
16. **Murray IR, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF.** Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO): Platelet-Rich Plasma and Mesenchymal Stem Cells. *J Bone Joint Surg [Am]* 2017;99-A:809–819.
17. **No authors listed.** The Cochrane Risk of Bias 2.0 Tool. A revised tool to assess risk of bias in randomized trials (RoB 2.0). http://training.cochrane.org/resource/rob-20-webinar (date last accessed 22 January 2017).
18. **Sterne JA, Hernán MA, Reeves BC, et al.** ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016;355:4919.
19. **Hooijmans CR, Leenaars M, Ritskes-Hoitinga M.** A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. *Altern Lab Anim* 2010;38:167–182.
20. **Fu SC, Cheuk YC, Yung SH, Rolf CG, Chan KM.** Systematic review of biological modulation of healing in anterior cruciate ligament reconstruction. *Orthop J Sports Med* 2014;2:2325967114526687.
21. **Teng C, Zhou CH, Xu D, Bi F.** Combination of platelet-rich plasma and bone marrow mesenchymal stem cells enhances tendon-bone healing in a rabbit model of anterior cruciate ligament reconstruction. *J Orthop Surg* 2016;11:96.

22. **Leong NL, Kabir N, Arshi A, et al.** Evaluation of polycaprolactone scaffold with basic fibroblast growth factor and fibroblasts in an athymic rat model for anterior cruciate ligament reconstruction. *Tissue Eng Part A* 2015;21:1859–1868.
23. **Hensler D, Illingworth KD, Musahl V, et al.** Does fibrin clot really enhance graft healing after double-bundle ACL reconstruction in a caprine model? *Knee Surg Sports Traumatol Arthrosc* 2015;23:669–679.
24. **Fleming BC, Proffen BL, Vavken P, et al.** Increased platelet concentration does not improve functional graft healing in bio-enhanced ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc* 2015;23:1161–1170.
25. **Li X, He JK, Bian W, et al.** A novel silk-based artificial ligament and tricalcium phosphate/polyether ether ketone anchor for anterior cruciate ligament reconstruction - safety and efficacy in a porcine model. *Acta Biomater* 2014;10:3696–3704.
26. **Weimin P, Dan L, Yiyong W, Yunyu H, Li Z.** Tendon-to-bone healing using an injectable calcium phosphate cement combined with bone xenograft/BMP composite. *Biomaterials* 2013;34:9926–9936.
27. **Murray MM, Fleming BC.** Use of a bioactive scaffold to stimulate anterior cruciate ligament healing also minimizes posttraumatic osteoarthritis after surgery. *Am J Sports Med* 2013;41:1762–1770.
28. **Lee AJ, Chung WH, Kim DH, et al.** Anterior cruciate ligament reconstruction in a rabbit model using canine small intestinal submucosa and autologous platelet-rich plasma. *J Surg Res* 2012;178:206–215.
29. **Chen J, Yang L, Guo L, Duan X.** Sodium hyaluronate as a drug-release system for VEGF 165 improves graft revascularization in anterior cruciate ligament reconstruction in a rabbit model. *Exp Ther Med* 2012;4:430–434.
30. **Zhang W, Pan W, Zhang M, Wei Y.** In vivo evaluation of two types of bioactive scaffold used for tendon-bone interface healing in the reconstruction of anterior cruciate ligament. *Biotechnol Lett* 2011;33:837–844.
31. **Wei X, Mao Z, Hou Y, et al.** Local administration of TGFβ-1/VEGF165 gene-transduced bone mesenchymal stem cells for Achilles allograft replacement of the anterior cruciate ligament in rabbits. *Biochem Biophys Res Commun* 2011;406:204–210.
32. **Pan W, Wei Y, Zhou L, Li D.** Comparative in vivo study of injectable biomaterials combined with BMP for enhancing tendon graft osteointegration for anterior cruciate ligament reconstruction. *J Orthop Res* 2011;29:1015–1021.
33. **Hashimoto Y, Naka Y, Fukunaga K, Nakamura H, Takaoka K.** ACL reconstruction using bone-tendon-bone graft engineered from the semitendinosus tendon by injection of recombinant BMP-2 in a rabbit model. *J Orthop Res* 2011;29:1923–1930.
34. **Nakase J, Kitaoka K, Matsumoto K, Tomita K.** Facilitated tendon-bone healing by local delivery of recombinant hepatocyte growth factor in rabbits. *Arthroscopy* 2010;26:84–90.
35. **Lu Y, Markel MD, Nemke B, et al.** Influence of hydroxyapatite-coated and growth factor-releasing interference screws on tendon-bone healing in an ovine model. *Arthroscopy* 2009;25:1427–1434.
36. **Sasaki K, Kuroda R, Ishida K, et al.** Enhancement of tendon-bone osteointegration of anterior cruciate ligament graft using granulocyte colony-stimulating factor. *Am J Sports Med* 2008;36:1519–1527.
37. **Ma CB, Kawamura S, Deng XH, et al.** Bone morphogenetic proteins-signaling plays a role in tendon-to-bone healing: a study of rhBMP-2 and noggin. *Am J Sports Med* 2007;35:597–604.
38. **Yoshikawa T, Tohyama H, Katsura T, Kondo E, Kotani Y, Matsumoto H, et al.** Effects of local administration of vascular endothelial growth factor on mechanical characteristics of the semitendinosus tendon graft after anterior cruciate ligament reconstruction in sheep. *Am J Sports Med* 2006;34:1918–1925.
39. **Yamazaki S, Yasuda K, Tomita F, Tohyama H, Minami A.** The effect of transforming growth factor-beta1 on intraosseous healing of flexor tendon autograft replacement of anterior cruciate ligament in dogs. *Arthroscopy* 2005;21:1034–1041.
40. **Yasuda K, Tomita F, Yamazaki S, Minami A, Tohyama H.** The effect of growth factors on biomechanical properties of the bone-patellar tendon-bone graft after anterior cruciate ligament reconstruction: a canine model study. *Am J Sports Med* 2004;32:870–880.
41. **Weiler A, Förster C, Hunt P, et al.** The influence of locally applied platelet-derived growth factor-BB on free tendon graft remodeling after anterior cruciate ligament reconstruction. *Am J Sports Med* 2004;32:881–891.
42. **Anderson K, Seneviratne AM, Izawa K, et al.** Augmentation of tendon healing in an intraarticular bone tunnel with use of a bone growth factor. *Am J Sports Med* 2001;29:689–698.
43. **Mihelic R, Pecina M, Jelic M, et al.** Bone morphogenetic protein-7 (osteogenic protein-1) promotes tendon graft integration in anterior cruciate ligament reconstruction in sheep. *Am J Sports Med* 2004;32:1619–1625.
44. **Li H, Chen C, Ge Y, Chen S.** Spray-painted human fibronectin coating as an effective strategy to enhance graft ligamentization of a polyethylene terephthalate artificial ligament. *Biotechnol Lett* 2014;36:1079–1088.
45. **Del Torto M, Enea D, Panfoli N, et al.** Hamstrings anterior cruciate ligament reconstruction with and without platelet rich fibrin matrix. *Knee Surg Sports Traumatol Arthrosc* 2015;23:3614–3622.
46. **Valentí Azcárate A, Lamo-Espinosa J, Aquerreta Beola JD, et al.** Comparison between two different platelet-rich plasma preparations and control applied during anterior cruciate ligament reconstruction. Is there any evidence to support their use? *Injury* 2014;45(Suppl 4):S36–S41.
47. **Vadalà A, Iorio R, De Carli A, et al.** Platelet-rich plasma: does it help reduce tunnel widening after ACL reconstruction? *Knee Surg Sports Traumatol Arthrosc* 2013;21:824–829.
48. **Seijas R, Ares O, Catala J, et al.** Magnetic resonance imaging evaluation of patellar tendon graft remodelling after anterior cruciate ligament reconstruction with or without platelet-rich plasma. *J Orthop Surg (Hong Kong)* 2013;21:10–14.
49. **Rupreht M, Vogrin M, Hussein M.** MRI evaluation of tibial tunnel wall cortical bone formation after platelet-rich plasma applied during anterior cruciate ligament reconstruction. *Radiol Oncol* 2013;47:119–124.
50. **Mirzatolooei F, Alamdari MT, Khalkhali HR.** The impact of platelet-rich plasma on the prevention of tunnel widening in anterior cruciate ligament reconstruction using quadrupled autologous hamstring tendon: a randomised clinical trial. *Bone Joint J* 2013;95-B:65–69.
51. **Darabos N, Haspl M, Moser C, et al.** Intraarticular application of autologous conditioned serum (ACS) reduces bone tunnel widening after ACL reconstructive surgery in a randomized controlled trial. *Knee Surg Sports Traumatol Arthrosc* 2011;19(Suppl 1):S36–S46.
52. **Vogrin M, Rupreht M, Crnjac A, et al.** The effect of platelet-derived growth factors on knee stability after anterior cruciate ligament reconstruction: a prospective randomized clinical study. *Wien Klin Wochenschr* 2010;122(Suppl 2):91–95.
53. **Sánchez M, Anitua E, Azofra J, et al.** Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. *Arthroscopy* 2010;26:470–480.
54. **Radice F, Yánez R, Gutiérrez V, et al.** Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. *Arthroscopy* 2010;26:50–57.
55. **Figueroa D, Melean P, Calvo R, et al.** Magnetic resonance imaging evaluation of the integration and maturation of semitendinosus-gracilis graft in anterior cruciate ligament reconstruction using autologous platelet concentrate. *Arthroscopy* 2010;26:1318–1325.
56. **Silva A, Sampaio R.** Anatomic ACL reconstruction: does the platelet-rich plasma accelerate tendon healing? *Knee Surg Sports Traumatol Arthrosc* 2009;17:676–682.
57. **Nin JR, Gasque GM, Azcárate AV, Beola JD, Gonzalez MH.** Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy* 2009;25:1206–1213.
58. **Orrego M, Larrain C, Rosales J, et al.** Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. *Arthroscopy* 2008;24:1373–1380.
59. **Ventura A, Terzaghi C, Borgo E, et al.** Use of growth factors in ACL surgery: Preliminary study. *J Orthop Traumatol* 2005;6:76–79.
60. **Pan W, Hu Y, Wei Y, et al.** Recombined bone xenografts enhance tendon graft osteointegration of anterior cruciate ligament reconstruction. *Int Orthop* 2009;33:1761–1768.
61. **Jin SK, Lee JH, Hong JH, et al.** Enhancement of Osseointegration of Artificial Ligament by Nano-Hydroxyapatite and Bone Morphogenic Protein-2 into the Rabbit Femur. *Tissue Eng Regen Med* 2016;13:284–296.
62. **Zhai W, Lv C, Zheng Y, et al.** Weak link of tendon-bone healing and a control experiment to promote healing. *Arch Orthop Trauma Surg* 2013;133:1533–1541.
63. **Sun X, Liu W, Cheng G, et al.** The influence of connective tissue growth factor on rabbit ligament injury repair. *Bone Joint Res* 2017;6:399–404.
64. **Andriolo L, Di Matteo B, Kon E, et al.** PRP Augmentation for ACL reconstruction. *BioMed Res Int* 2015;2015:371746.
65. **Schippinger G, Prüller F, Divjak M, et al.** Autologous platelet-rich plasma preparations: influence of nonsteroidal anti-inflammatory drugs on platelet function. *Orthop J Sports Med* 2015;3:2325967115588896.
66. **Wang J, Xu J, Fu W, et al.** Biodegradable magnesium screws accelerate fibrous tissue mineralization at the tendon-bone insertion in anterior cruciate ligament reconstruction model of rabbit. *Sci Rep* 2017;7:40369.
67. **Cheng P, Han P, Zhao C, et al.** Magnesium inference screw supports early graft incorporation with inhibition of graft degradation in anterior cruciate ligament reconstruction. *Sci Rep* 2016;6:26434.
68. **Chou YC, Yeh WL, Chao CL, et al.** Enhancement of tendon-bone healing via the combination of biodegradable collagen-loaded nanofibrous membranes and a three-dimensional printed bone-anchoring bolt. *Int J Nanomedicine* 2016;11:4173–4186.
69. **Kawai T, Yamada T, Yasukawa A, et al.** Anterior cruciate ligament reconstruction using chitin-coated fabrics in a rabbit model. *Artif Org* 2010;34:55–64.

70. Li H, Chen S, Wu Y, et al. Enhancement of the osseointegration of a polyethylene terephthalate artificial ligament graft in a bone tunnel using 58S bioglass. *Int Orthop* 2012;36:191–197.

71. Cho S, Li H, Chen C, et al. Cationised gelatin and hyaluronic acid coating enhances polyethylene terephthalate artificial ligament graft osseointegration in porcine bone tunnels. *Int Orthop* 2013;37:507–513.

72. Vaquette C, Viateau V, Guérard S, et al. The effect of polystyrene sodium sulfonate grafting on polyethylene terephthalate artificial ligaments on in vitro mineralisation and in vivo bone tissue integration. *Biomaterials* 2013;34:7048–7063.

73. Bi F, Shi Z, Liu A, Guo P, Yan S. Anterior cruciate ligament reconstruction in a rabbit model using silk-collagen scaffold and comparison with autograft. *PLoS One* 2015;10:0125900.

74. Shen W, Chen X, Hu Y, et al. Long-term effects of knitted silk-collagen sponge scaffold on anterior cruciate ligament reconstruction and osteoarthritis prevention. *Biomaterials* 2014;35:8154–8163.

75. Han F, Zhang P, Sun Y, et al. Hydroxyapatite-doped polycaprolactone nanofiber membrane improves tendon-bone interface healing for anterior cruciate ligament reconstruction. *Int J Nanomedicine* 2015;10:7333–7343.

76. Hsu SL, Wang CJ. The use of demineralized bone matrix for anterior cruciate ligament reconstruction: a radiographic, histologic, and immunohistochemical study in rabbits. *J Surg Res* 2014;187:219–224.

77. Weimin P, Dan L, Yiyong W, Yunyu H, Li Z. Tendon-to-bone healing using an injectable calcium phosphate cement combined with bone xenograft/BMP composite. *Biomaterials* 2013;34:9926–9936.

78. Lovric V, Chen D, Yu Y, et al. Effects of demineralized bone matrix on tendon-bone healing in an intra-articular rodent model. *Am J Sports Med* 2012;40:2365–2374.

79. Kadonishi Y, Deie M, Takata T, Ochi M. Acceleration of tendon-bone healing in anterior cruciate ligament reconstruction using an enamel matrix derivative in a rat model. *J Bone Joint Surg [Br]* 2012;94-B:205–209.

80. Gulotta LV, Kovacevic D, Ying L, et al. Augmentation of tendon-to-bone healing with a magnesium-based bone adhesive. *Am J Sports Med* 2008;36:1290–1297.

81. Elnikety S, Pendegrass CJ, de Godoy RF, Holden C, Blunn GW. Augmentation and repair of tendons using demineralised cortical bone. *BMC Musculoskelet Disord* 2016;17:483.

82. Sundar S, Pendegrass CJ, Blunn GW. Tendon bone healing can be enhanced by demineralized bone matrix: a functional and histological study. *J Biomed Mater Res B Appl Biomater* 2009;88:115–122.

83. Thangarajah T, Henshaw F, Sanghani-Kerai A, et al. The effectiveness of demineralized cortical bone matrix in a chronic rotator cuff tear model. *J Shoulder Elbow Surg* 2017;26:619–626.

84. Thangarajah T, Shahbazi S, Pendegrass CJ, et al. Tendon Reattachment to Bone in an Ovine Tendon Defect Model of Retraction Using Allogenic and Xenogenic Demineralised Bone Matrix Incorporated with Mesenchymal Stem Cells. *PloS One* 2016;11:0161473.

85. Iorio R, Di Sanzo V, Vadalà A, et al. Nanohydroxyapatite-based bone graft substitute in tunnel enlargement after ACL surgery: RMN study. *Clin Ter* 2013;164:101–106.

86. Mutsuzaki H, Fujie H, Nakajima H, et al. Effect of calcium phosphate-hybridized tendon graft in anatomic single-bundle ACL reconstruction in goats. *Orth J Sports Med* 2016;4:2325967116662653.

87. Kuang GM, Yau WP, Lu WW, Chiu KY. Local application of strontium in a calcium phosphate cement system accelerates healing of soft tissue tendon grafts in anterior cruciate ligament reconstruction experiment using a rabbit model. *Am J Sports Med* 2014;42:2996–3002.

88. Kuang GM, Yau WP, Lu WW, Chiu KY. Use of a strontium-enriched calcium phosphate cement in accelerating the healing of soft-tissue tendon graft within the bone tunnel in a rabbit model of anterior cruciate ligament reconstruction. *Bone Joint J* 2013;95-B:923–928.

89. Mutsuzaki H, Kanamori A, Ikeda K, et al. Effect of calcium phosphate-hybridized tendon graft in anterior cruciate ligament reconstruction: a randomized controlled trial. *Am J Sports Med* 2012;40:1772–1780.

90. Mutsuzaki H, Sakane M, Fujie H, et al. Effect of calcium phosphate-hybridized tendon graft on biomechanical behavior in anterior cruciate ligament reconstruction in a goat model: novel technique for improving tendon-bone healing. *Am J Sports Med* 2011;39:1059–1066.

91. Shen H, Qiao G, Cao H, Jiang Y. An histological study of the influence of osteoinductive calcium phosphate ceramics on tendon healing pattern in a bone tunnel with suspensory fixation. *Int Orthop* 2010;34:917–924.

92. Wen CY, Qin L, Lee KM, Chan KM. The use of brushite calcium phosphate cement for enhancement of bone-tendon integration in an anterior cruciate ligament reconstruction rabbit model. *J Biomed Mater Res B Appl Biomater* 2009;89:466–474.

93. Huangfu X, Zhao J. Tendon-bone healing enhancement using injectable tricalcium phosphate in a dog anterior cruciate ligament reconstruction model. *Arthroscopy* 2007;23:455–462.

94. Tien YC, Chih TT, Lin JHC, Ju CP, Lin SD. Augmentation of tendon-bone healing by the use of calcium-phosphate cement. *J Bone Joint Surg [Br]* 2004;86-B:1072–1076.

95. Mutsuzaki H, Sakane M, Nakajima H, et al. Calcium-phosphate-hybridized tendon directly promotes regeneration of tendon-bone insertion. *J Biomed Mater Res A* 2004;70:319–327.

96. Teuschl A, Heimel P, Nürnberger S, et al. A Novel Silk Fiber-Based Scaffold for Regeneration of the Anterior Cruciate Ligament: Histological Results From a Study in Sheep. *Am J Sports Med* 2016;44:1547–1557.

97. Kouroupis D, Kyrkou A, Triantafyllidi E, et al. Generation of stem cell-based bioartificial anterior cruciate ligament (ACL) grafts for effective ACL rupture repair. *Stem Cell Res (Amst)* 2016;17:448–457.

98. Kosaka M, Nakase J, Hayashi K, Tsuchiya H. Adipose-Derived Regenerative Cells Promote Tendon-Bone Healing in a Rabbit Model. *Arthroscopy* 2016;32:851–859.

99. Li H, Fan J, Sun L, et al. Functional regeneration of ligament-bone interface using a triphasic silk-based graft. *Biomaterials* 2016;106:180–192.

100. Zhu JX, Zhang X, Shao ZX, et al. In vivo study of ligament-bone healing after anterior cruciate ligament reconstruction using autologous tendons with mesenchymal stem cells affinity peptide conjugated electrospun nanofibrous scaffold. *J Nanomaterials* 2013.

101. Jang KM, Lim HC, Jung WY, Moon SW, Wang JH. Efficacy and safety of human umbilical cord blood-derived mesenchymal stem cells in anterior cruciate ligament reconstruction of a rabbit model: new strategy to enhance tendon graft healing. *Arthroscopy* 2015;31:1530–1539.

102. Lui PPY, Wong OT, Lee YW. Application of tendon-derived stem cell sheet for the promotion of graft healing in anterior cruciate ligament reconstruction. *Am J Sports Med* 2014;42:681–689.

103. Mifune Y, Matsumoto T, Takayama K, et al. Tendon graft revitalization using adult anterior cruciate ligament (ACL)-derived CD34+ cell sheets for ACL reconstruction. *Biomaterials* 2013;34:5476–5487.

104. Mifune Y, Matsumoto T, Ota S, et al. Therapeutic potential of anterior cruciate ligament-derived stem cells for anterior cruciate ligament reconstruction. *Cell Transplant* 2012;21:1651–1665.

105. Fan H, Liu H, Toh SL, Goh JC. Anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold in large animal model. *Biomaterials* 2009;30:4967–4977.

106. Fan H, Liu H, Wong EJW, Toh SL, Goh JCH. In vivo study of anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold. *Biomaterials* 2008;29:3324–3337.

107. Soon MY, Hassan A, Hui JH, Goh JC, Lee E. An analysis of soft tissue allograft anterior cruciate ligament reconstruction in a rabbit model: a short-term study of the use of mesenchymal stem cells to enhance tendon osteointegration. *Am J Sports Med* 2007;35:962–971.

108. Lim JK, Hui J, Li L, et al. Enhancement of tendon graft osteointegration using mesenchymal stem cells in a rabbit model of anterior cruciate ligament reconstruction. *Arthroscopy* 2004;20:899–910.

109. Silva A, Sampaio R, Fernandes R, Pinto E. Is there a role for adult non-cultivated bone marrow stem cells in ACL reconstruction? *Knee Surg Sports Traumatol Arthrosc* 2014;22:66–71.

110. Martinek V, Latterman C, Usas A, et al. Enhancement of tendon-bone integration of anterior cruciate ligament grafts with bone morphogenetic protein-2 gene transfer: a histological and biomechanical study. *J Bone Joint Surg [Am]* 2002;84-A:1123–1131.

111. Wang CJ, Weng LH, Hsu SL, et al. pCMV-BMP-2-transfected cell-mediated gene therapy in anterior cruciate ligament reconstruction in rabbits. *Arthroscopy* 2010;26:968–976.

112. Zhang X, Ma Y, Fu X, et al. Runx2-Modified Adipose-Derived Stem Cells Promote Tendon Graft Integration in Anterior Cruciate Ligament Reconstruction. *Sci Rep* 2016;6:19073.

113. Kawakami Y, Takayama K, Matsumoto T, et al. Anterior cruciate ligament-derived stem cells transduced with BMP2 accelerate graft-bone integration after ACL reconstruction. *Am J Sports Med* 2017;45:584–597.

114. Chen BA, Li B, Qi YJ, et al. Enhancement of tendon-to-bone healing after anterior cruciate ligament reconstruction using bone marrow-derived mesenchymal stem cells genetically modified with bFGF/BMP2. *Sci Rep* 2016;6:25940.

115. Takayama K, Kawakami Y, Mifune Y, et al. The effect of blocking angiogenesis on anterior cruciate ligament healing following stem cell transplantation. *Biomaterials* 2015;60:9–19.

116. Dong Y, Zhang Q, Li Y, Jiang J, Chen S. Enhancement of tendon-bone healing for anterior cruciate ligament (ACL) reconstruction using bone marrow-derived mesenchymal stem cells infected with BMP-2. *Int J Mol Sci* 2012;13:13605–13620.

117. Li F, Jia H, Yu C. ACL reconstruction in a rabbit model using irradiated Achilles allograft seeded with mesenchymal stem cells or PDGF-B gene-transfected mesenchymal stem cells. *Knee Surg Sports Traumatol Arthrosc* 2007;15:1219–1227.

118. Hao ZC, Wang SZ, Zhang XJ, Lu J. Stem cell therapy: a promising biological strategy for tendon-bone healing after anterior cruciate ligament reconstruction. *Cell Prolif* 2016;49:154–162.

119. Matsumoto T, Ingham SM, Mifune Y, et al. Isolation and characterization of human anterior cruciate ligament-derived vascular stem cells. *Stem Cells Dev* 2012;21:859–872.

120. Li H, Chen J, Chen S. Remnant Repair-enhanced Polyethylene Terepthalate Artificial Ligament Graft Ligamentization. *Int J Sports Med* 2015;36:1015–1020.

121. Song GY, Zhang J, Li X, et al. Acute anterior cruciate ligament reconstruction with an augmented remnant repair: a comparative macroscopic and biomechanical study in an animal model. *Arthroscopy* 2014;30:344–351.

122. Sun L, Wu B, Tian M, Liu BC, Luo Y. Comparison of graft healing in anterior cruciate ligament reconstruction with and without a preserved remnant in rabbits. *Knee* 2013;20:537–544.

123. Sun L, Hou C, Wu B, Tian M, Zhou X. Effect of muscle preserved on tendon graft on intra-articular healing in anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc* 2013;21:1862–1868.

124. Takahashi T, Kondo E, Yasuda K, et al. Effects of remnant tissue preservation on the tendon graft in anterior cruciate ligament reconstruction: a biomechanical and histological study. *Am J Sports Med* 2016;44:1708–1716.

125. Song GY, Zhang J, Li X, Li Y, Feng H. Biomechanical and biological findings between acute anterior cruciate ligament reconstruction with and without an augmented remnant repair: a comparative in vivo animal study. *Arthroscopy* 2016;32:307–319.

126. Matsumoto T, Kubo S, Sasaki K, et al. Acceleration of tendon-bone healing of anterior cruciate ligament graft using autologous ruptured tissue. *Am J Sports Med* 2012;40:1296–1302.

127. Matsumoto T, Kuroda R, Matsushita T, et al. Reduction of tunnel enlargement with use of autologous ruptured tissue in anterior cruciate ligament reconstruction: a pilot clinical trial. *Arthroscopy* 2014;30:468–474.

128. Uddströmer L, Ritsilä V. Osteogenic capacity of periosteal grafts: a qualitative and quantitative study of membranous and tubular bone periosteum in young rabbits. *Scand J Plast* 1978;12:207–214.

129. Karaoglu S, Celik C, Korkusuz P. The effects of bone marrow or periosteum on tendon-to-bone tunnel healing in a rabbit model. *Knee Surg Sports Traumatol Arthrosc* 2009;17:170–178.

130. Chen CH, Chen WJ, Shih CH. Enveloping of periosteum on the hamstring tendon graft in anterior cruciate ligament reconstruction. *Arthroscopy* 2002;18:27E.

131. Dai Z, Bao W, Li S, et al. Enhancement of polyethylene terephthalate artificial ligament graft osseointegration using a periosteum patch in a goat model. *Int J Sports Med* 2016;37:493–499.

132. Chen C- H, Chang C- H, Su C- I, et al. Arthroscopic single-bundle anterior cruciate ligament reconstruction with periosteum-enveloping hamstring tendon graft: clinical outcome at 2 to 7 years. *Arthroscopy* 2010;26:907–917.

133. Chen C- H, Chen W- J, Shih C- H, Chou S- W. Arthroscopic anterior cruciate ligament reconstruction with periosteum-enveloping hamstring tendon graft. *Knee Surg Sports Traumatol Arthrosc* 2004;12:398–405.

134. Chen C- H, Chen W- J, Shih C- H. Enveloping of periosteum on the hamstring tendon graft in anterior cruciate ligament reconstruction. *Arthroscopy* 2002;18:27E.

135. Demirag B, Sarisozen B, Ozer O, Kaplan T, Ozturk C. Enhancement of tendon-bone healing of anterior cruciate ligament grafts by blockage of matrix metalloproteinases. *J Bone Joint Surg [Am]* 2005;87-A:2401–2410.

136. Lui PPY, Lee YW, Mok TY, Cheuk YC. Local administration of alendronate reduced peri-tunnel bone loss and promoted graft-bone tunnel healing with minimal systemic effect on bone in contralateral knee. *J Orthop Res* 2013;31:1897–1906.

137. Lui PP, Lee YW, Mok TY, Cheuk YC, Chan KM. Alendronate reduced peri-tunnel bone loss and enhanced tendon graft to bone tunnel healing in anterior cruciate ligament reconstruction. *Eur Cell Mater* 2013;25:78–96.

138. Bi F, Shi Z, Jiang S, Guo P, Yan S. Intermittently administered parathyroid hormone [1-34] promotes tendon-bone healing in a rat model. *Int J Mol Sci* 2014;15:17366–17379.

139. Oka S, Matsumoto T, Kubo S, et al. Local administration of low-dose simvastatin-conjugated gelatin hydrogel for tendon–bone healing in anterior cruciate ligament reconstruction. *Tissue Eng Part A* 2013;19:1233–1243.

140. Zhang P, Han F, Li Y, et al. Local delivery of controlled-release simvastatin to improve the biocompatibility of polyethylene terephthalate artificial ligaments for reconstruction of the anterior cruciate ligament. *Int J Nanomedicine* 2016;11:465–478.

141. Yeh WL, Lin SS, Yuan LJ, et al. Effects of hyperbaric oxygen treatment on tendon graft and tendon-bone integration in bone tunnel: biochemical and histological analysis in rabbits. *J Orthop Res* 2007;25:636–645.

142. Walsh WR, Stephens P, Vizesi F, et al. Effects of low-intensity pulsed ultrasound on tendon-bone healing in an intra-articular sheep knee model. *Arthroscopy* 2007;23:197–204.

143. Papatheodorou LK, Malizos KN, Poultsides LA, et al. Effect of transosseous application of low-intensity ultrasound at the tendon graft-bone interface healing: gene expression and histological analysis in rabbits. *Ultrasound Med Biol* 2009;35:576–584.

144. Wang CJ, Wang FS, Yang KD, et al. The effect of shock wave treatment at the tendon-bone interface-an histomorphological and biomechanical study in rabbits. *J Orthop Res* 2005;23:274–280.

145. Wang CJ, Ko JY, Chou WY, et al. Shockwave therapy improves anterior cruciate ligament reconstruction. *J Surg Res* 2014;188:110–118.

146. Kiapour A, Murray M. Basic science of anterior cruciate ligament injury and repair. *Bone Joint Res* 2014;3:20–31.

147. Muller-Borer BJ, Collins MC, Gunst PR, Cascio WE, Kypson AP. Quantum dot labeling of mesenchymal stem cells. *J Nanobiotechnology* 2007;5:9.

148. Hexter AT, Pendegrass C, Haddad F, Blunn G. Demineralized bone matrix to augment tendon-bone healing: a systematic review. *Orthop J Sports Med* 2017;5:2325967117734517.

149. Pan W, Cao Z, Li D, Zhang M. Evaluation of the potential application of three different biomaterials combined with bone morphological proteins for enhancing tendon-bone integration. *Injury* 2013;44:550–557.

Author contributions:
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G. Blunn: Helped write the manuscript and helped with the literature search
F. S. Haddad: Senior author, Supervision of the planning and writing of the manuscript, Reviewed the manuscript prior to submission.

Funding statement:
No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

This article was primary edited by A. D. Liddle and first proof edited by G. Scott.