

Systematic Review of Biological Modulation of Healing in Anterior Cruciate Ligament Reconstruction

Sai-Chuen Fu,^{*†} PhD, Yau-Chuk Cheuk,^{*†} MPhil, Shu-Hang Yung,^{*†} FRCS Ed (Orth), Christer Gustav Rolf,[‡] PhD, and Kai-Ming Chan,^{*†§} MCh (Orth), FRCS

Investigation performed at the Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Background: Whether biological modulation is effective to promote healing in anterior cruciate ligament (ACL) reconstruction remains unclear.

Purpose: To perform a systematic review of both clinical and experimental evidence of preclinical animal studies on biological modulation to promote healing in ACL reconstruction.

Study Design: Systematic review; Level of evidence, 2.

Methods: A systematic search was performed using the PubMed, Ovid, and Scopus search engines. Inclusion criteria were clinical and animal studies involving subjects with ACL injury with the use of biological modulation to promote healing outcomes. Methodological quality of clinical studies was evaluated using the Critical Appraisal Skill Programme (CASP) appraisal tool, and animal studies were evaluated by a scoring system based on a published checklist of good animal studies.

Results: Ten clinical studies and 50 animal studies were included. Twenty-five included studies were regarded as good quality, with a methodological score ≥ 5 . These studies suggested that transforming growth factor- β (TGF- β), mesenchymal stem cells, osteogenic factors, and modalities that reduce local inflammation may be beneficial to promote graft healing in ACL reconstruction.

Conclusion: This systematic review suggests that biological modulation is able to promote healing on top of surgical treatment for ACL injuries. This treatment strategy chiefly works through promotion of healing at the tunnel-graft interface, but the integrity of the intra-articular midsubstance of the graft would be another target for biological modulation.

Keywords: anterior cruciate ligament; growth factor; cell therapy; biological modulation

Currently, there are myriad studies investigating improvements in surgical methods for anterior cruciate ligament reconstruction (ACLR), for example, bone tunnel construction and placement, graft choices, graft preparation, and

graft fixation.^{3,19,21} Yet, perfect surgical techniques still need an adequate biological healing response to yield good clinical outcomes. Reports have attributed poor graft healing as one of the causes leading to nontraumatic ACLR failure.^{5,15} Graft healing of ACLR involves slow biological processes,¹⁸ which are primarily attributed to graft remodeling such as intratunnel graft incorporation and intra-articular graft ligamentization.⁵⁰ Previous approaches to enhance the healing at the graft-tunnel interface include the use of mesenchymal stem cells, growth factors, biomaterials, or biophysical intervention. Most of these preclinical animal studies still need further scrutiny to see if further clinical trials were worthwhile. An overall picture of the current development on biological modulation for ACLR would be obtained by performing a systematic review of both clinical and preclinical evidence. By critical appraisal on the study quality and significance of these studies, a better understanding on the effectiveness of biological modulation on ACLR can be attained. A summary of the

[§]Address correspondence to Kai-Ming Chan, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong (e-mail: kaimingchan@cuhk.edu.hk).

^{*}Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China.

[†]Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China.

[‡]Department of Orthopaedic Surgery, Huddinge University Hospital, CLINTEC, Karolinska Institutet, Stockholm, Sweden.

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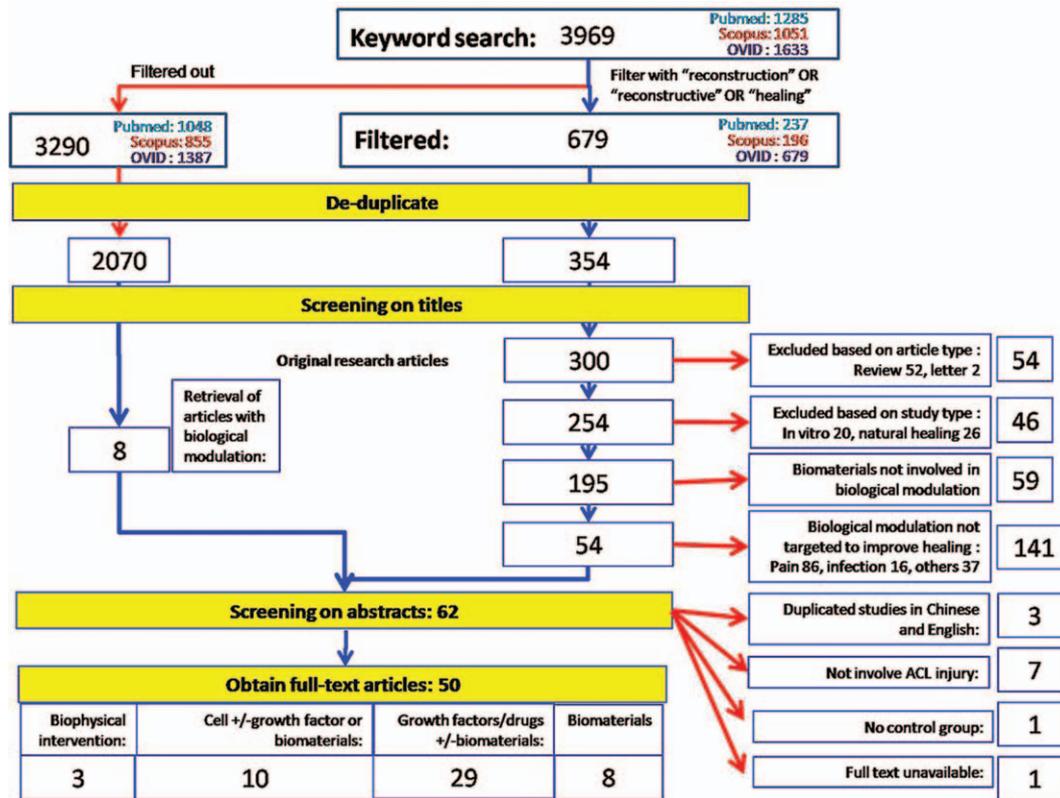


Figure 1. Flowchart showing results of database search, article elimination, and final inclusion performed in January 2013. Results of the updated search performed in January 2014 are reported separately in the Results section.

proposed mechanisms of the treatment effect may also provide insights for further development of new approaches of biological modulation for ACLR.

The purposes of this study were (1) to perform a systematic review on the effectiveness of biological modulation to promote healing in ACLR, (2) to evaluate different biological modulations based on the proposed mechanisms, and (3) to evaluate the clinical significance of preclinical animal studies on biological modulation of ACLR.

METHODS

Study Selection

The inclusion criteria for studies in this systematic review consisted of the following:

- **Study types:** Clinical studies and animal studies. Studies included in searched reviews or systematic reviews were also tracked.
- **Study group:** Patients with ACL tears who received surgical treatment; animal models with ACL injuries.
- **Intervention type:** Drugs, growth factors, cells, interface biomaterials to promote graft incorporation, and biophysical interventions that stimulate healing.
- **Language:** No restriction.

The exclusion criteria consisted of the following:

- **Study types:** Studies of natural healing without intervention, in vitro studies, and studies without a control group.
- **Article type:** Articles other than original research articles.
- **Intervention types:** Interventions not related to biological modulation to promote healing, such as surgical treatments (direct sutures, fixation methods), artificial grafts or screws that do not aim at biological modulation, anesthetics, analgesics, and rehabilitation protocols.

Search Strategies

Systematic searches were carried out in January 2013 using PubMed, Ovid, and Scopus databases. The keywords in combination with search operants were as follows: (ACL OR anterior cruciate ligament) AND (growth factor OR stem cell OR drug OR biomaterial OR biophysical intervention). A postsearch filtering with keywords *reconstruction*, *reconstructive*, and *healing* in the titles was used to facilitate the identification of relevant studies. Studies with biological modulation on ACL lesion without reconstructive surgery were also identified by screening the titles. The search results from 3 different databases were merged, and

TABLE 1
Assessment Criteria of Methodological Quality of Animal Studies of ACLR^a

Criteria	Score	Remarks
1. Unit of sample	Unilateral: 1 Bilateral: 0	Studies with bilateral operation may regard each limb as an independent sample and assign them to different treatment groups. Unless the sample unit was specified as number of animal instead of number of limbs, animal studies with unilateral operation with animal as sample unit will be better.
2. Standardization of surgical procedure	Yes: 1 No: 0	Standardization of surgical procedure includes the descriptions about graft harvest, approaching intra-articular region, drilling tunnels, graft tensioning, and fixation method. Studies with these descriptions would be regarded as standardized procedures as major surgical variables are controlled.
3. Description of postoperative complications and follow-up	Yes: 1 No: 0	Records of postoperative complications such as broken sutures, wound infection, and early death are regarded to have better study quality.
4. Report of failure mode in mechanical test	Yes: 1 No: 0	Since most ACLR animal studies used mechanical testing as the primary outcome, report of failure mode is important to reveal the quality and the implications of the mechanical tests.
5. Variation (ratio of SD to mean)	<50%: 1 >50%: 0	For quantitative measure, large standard deviation may imply poor precision or large intra-group variations, which is regarded to have lower study quality.
6. Statistical method	Appropriate: 1 Questionable: 0	Questionable statistical analyses include the use of unpaired test for paired samples, parametric test for ordinal data with a few ranks, the use of unadjusted multiple comparisons instead of ANOVA or Kruskal-Wallis test.
7. Description of selection region of interest	Yes: 1 No: 0	For histology/imaging outcome measure, description of systematic/random sampling of region of interest is considered to provide better study quality.
8. Semiquantitative scoring/image analysis	Yes: 1 No: 0	For histology/imaging outcome measure, implementation of scoring systems or image analysis protocol is considered to provide better study quality.

^aACLR, anterior cruciate ligament reconstruction; ANOVA, analysis of variance; SD, standard deviation.

duplicate studies were removed. Application of inclusion/exclusion criteria on the search results was started by screening the title and then the abstracts. The full text of the filtered articles was then obtained for data extraction, such as publication years, first author, type of modulation, animal model, sample size, follow-up time, key outcome measures, and major findings. A flowchart of the search results is shown in Figure 1. After the original search in January 2013, an updated search was conducted in January 2014 to cover publications from January 2013 to January 2014. The search criteria remained the same as the original search.

Assessment of Study Quality

Scientific evidence for effectiveness of biological modulation of healing of ACL injuries includes information from both clinical trials and preclinical animal studies. Clinical trials (level 1 or 2) are regarded as “high” evidence level, and the Critical Appraisal Skill Programme (CASP) appraisal form was used to evaluate the study quality. Animal studies are of lower evidence level, and they can be further stratified into 5 ranks based on outcome measures:

- A: Quantitative outcome measures analogous to clinical outcome measures (eg, knee laxity, activity level, and gait)
- B: Mechanical test of graft complex strength (ultimate load, linear stiffness) as quantitative outcome measures
- C: Biochemical measurement as quantitative outcome measures
- D: Semiquantitative imaging/histological assessment
- E: Qualitative imaging/histological assessment

The quality of animal studies was assessed according to the criteria adapted from the checklist of Hooijmans et al,²² provided in Table 1.

RESULTS

Search Results

In the original search, titles of 2424 studies were screened from the systematic searches; 2070 articles were filtered out with the absence of the keywords *reconstruction*, *reconstructive*, or *healing*. After applying exclusion criteria on the remaining 354 articles based on article type and study type, 254 articles were left. Fifty-nine studies included the use of biomaterials that did not involve biological modulation; 141 studies involved biological modulation that did not primarily promote healing (ie, pain relief, infection). Based on inclusion criteria, 54 studies were identified as clinical studies or animal studies that involved biological modulation to promote graft healing in ACLR. Screening the titles of the filtered 2070 articles further yielded 8 articles that might meet inclusion criteria. These studies used *ACL surgery*, *bone-tendon healing*, *wound*, *injury*, or *ACL* instead of *healing/reconstruction* in the titles. The abstracts of the 62 included articles were screened. Three studies were later excluded owing to duplication in Chinese and English literature with the same study design. Seven studies focused on biological modulation on intact ACLs and were thus excluded. One study was excluded because of a lack of a control group. One study was excluded since the full text was unavailable. Fifty studies were therefore included for data extraction and critical appraisal on study quality. In the

TABLE 2
Search Results of Studies Investigating Biological Modulation of Healing in ACL Reconstruction^a

Year/Author	Biological Modulation	Type	Human/Animal	n	Surgery/Graft	Follow-up, Min-Max	Outcome Measures	Finding	Evidence Level	Quality Score
2014/Silva et al ⁵⁴	Bone marrow concentrate	GF/drugs	Clinical	43	ACLR/HS, DB	3 mo	Imaging	No difference	2	4/10
2013/Zhang et al ⁷³	Autologous bone marrow	GF/drugs	Rabbit	32	ACLR/ ST	2 wk–12 wk	Histology, mech	Positive	B	5/8
2013/Zhai et al ⁶⁹	PRP and demineralized bone protein	GF/drugs	Rabbit	48	ACLR/ST	2 wk–4 wk–8 wk–12 wk	Histology, mech, imaging	Positive	B	5/8
2013/Xie et al ⁶⁵	PRP	GF/drugs	Dog	36	ACLR/Flex	2 wk–12 wk	Biochem	Positive	C	1/8
2013/Weimin et al ⁶²	Calcium phosphate cement with bone xenograft/BMP composite	GF/drugs	Rabbit	90	ACLR/EXT	6 wk–24 wk	Histology, mech, imaging	Positive	B	3/8
2013/Oka et al ⁴³	Simvastatin-conjugated gelatin hydrogel	GF/drugs	Rabbit	42	ACLR/ HS	2 wk–8 wk	Histology, mech, imaging	Positive	B	6/8
2013/Mifune et al ³⁹	ACL-derived cell sheet	Cell	Rat	27	ACLR/Flex	2 wk–8 wk	Histology, mech	Positive	B	4/8
2013/Lui et al ³⁶	Alendronate (systemic)	GF/drugs	Rat	84	ACLR/Flex	2 wk–6 wk	Histology, mech, imaging	Positive	B	7/8
2013/Lui et al ³⁵	Alendronate (local)	GF/drugs	Rat	72	ACLR/Flex	2 wk–6 wk	Histology, mech, imaging	Positive	B	7/8
2014/Hsu and Wang ²³	Demineralized bone matrix	Biomaterial	Rabbit	10	ACLR/EXT	4 wk–12 wk	Histology, imaging	Positive	D	5/8
2013/Cho et al ⁸	Cationized gelatin and hyaluronic acid coating	GF/drugs	Porcine	6	ACLR/PET	3 mo	Histology, imaging	Positive	D	5/8
2012/Shoji et al ⁵²	MicroRNA	GF/drugs	Rat	55	ACLT partial	1 wk–4 wk	Histology, mech, biochem	Positive	B	5/8
2012/Lee et al ³²	SIS/PRP	GF/drugs	Rabbit	20	ACLR/ braided SIS	1 wk–8 wk	Histology, mech, imaging	Negative	B	5/8
2012/Kadonishi et al ²⁶	Enamel	Biomaterial	Rat	30	ACLR/Flex	4 wk–12 wk	Histology, mech	Positive	B	2/8
2012/Ediz et al ¹⁴	Electrostimulation	Biophy	Clinical	29	ACLR/HS	1 d–6 mo	Clinical, quest	Positive	1	8/10
2012/Dong et al ¹²	BMSC-BMP2 transfected	Cell	Rabbit	30	ACLR/GAS	4 wk–8 wk	Histology, mech	Positive	B	4/8
2012/Chen et al ⁷	Periosteum progenitor cell sheet	Cell	Rabbit	20	ACLR/EXT	8 wk	Histology, mech	Positive	B	2/8
2012/Chen et al ⁶	VEGF in hyaluronan	GF/drugs	Rabbit	45	ACLR/BTB Allo	2 wk–8 wk	Histology, mech	Positive	B	4/8
2011/Zhang et al ⁷²	Bioactive scaffold	Biomaterial	Rabbit	51	ACLR/Flex	12 wk	Histology, mech, imaging	No difference	B	2/8
2011/Zhang et al ⁷¹	Ginseng	GF/drugs	Rabbit	20	ACLR/EXT	4 wk–8 wk	Histology	Positive	E	3/8
2011/Wei et al ⁶⁰	TGF-β/VEGF transfected MSC	Cell	Rabbit	176	ACLR/AT	3 wk–24 wk	Histology, mech	Positive	B	6/8
2011/Qin et al ⁴⁸	TGF-β plasmid matrix	GF/drugs	Rabbit	48	ACLR/ST	1 mo–6 mo	Histology	Positive	E	2/8
2011/Pan et al ⁴⁵	BMP + materials	GF/drugs	Rabbit	51	ACLR	2 wk–12 wk	Histology, mech, imaging	Positive	B	2/8
2011/Ni et al ⁴²	Calcium acid phosphate	Biomaterial	Dog	36	ACLR/HS	1 mo–6 mo	Histology, mech	Positive	B	1/8
2011/Mutsuzaki et al ⁴¹	Calcium phosphate	Biomaterial	Goat	18	ACLR/HS, Flex	1 y	Clinical, lax	Positive	A	7/8
2011/Kondo et al ³⁰	TGF-β–treated synovial cells	Cell	Sheep	52	ACLR/ST	1 wk–12 wk	Histology, mech	Positive	B	5/8
2011/Hashimoto et al ²⁰	BMP2 injection	GF/drugs	Rabbit	40	ACLR/ST	4 wk–8 wk	Histology, mech, imaging	Positive	B	4/8
2011/Darabos et al ⁹	Autologous conditioned serum	GF/drugs	Clinical	62	ACLR/HS, BTB	10 d–12 mo	Imaging, quest, biochem	Positive	1	8/10
2010/Wang et al ⁵⁹	BMP-transfected cells	Cell	Rabbit	36	ACLR/EXT	1 wk–12 wk	Histology, mech, imaging	Positive	B	6/8
2010/Vogrin et al ⁵⁸	PRP	GF/drugs	Clinical	50	ACLR/HS	3 mo–6 mo	Lax	Positive	1	6/10
2010/Shen et al ⁵¹	Calcium phosphate ceramics	Biomaterial	Rabbit	30	ACLR/ST	4 wk–12 wk	Histology	Positive	E	2/8
2009/Wen et al ⁶³	Bone cement	Biomaterial	Rabbit	28	ACLR/Flex	6 wk–12 wk	Histology, mech, imaging	Positive	B	2/8
2009/Silva et al ⁵³	PRP	GF/drugs	Clinical	40	ACLR/HS, DB	3 mo	Imaging	No difference	2	1/10
2009/Papatheodorou et al ⁴⁶	LIPUS	Biophy	Rabbit	52	ACLR/EXT	1 d–21 d	Biochem, histology	Positive	C	3/8
2008/Zhang et al ⁷⁰	bFGF	GF/drugs	Dog	14	ACLR/EXT, Allo	1 wk–6 wk	Histology	Positive	E	1/8
2008/Sasaki et al ⁴⁹	Granulocyte stimulating factor	GF/drugs	Dogs	28	ACLR/Flex	2 wk–4 wk	Histology, mech, biochem, imaging	Positive	B	2/8

(continued)

TABLE 2 (continued)

Year/Author	Biological Modulation	Type	Human/Animal	n	Surgery/Graft	Follow-up, Min-Max	Outcome Measures	Finding	Evidence Level	Quality Score
2008/Orrago et al ⁴⁴	Platelet concentrate + bone plug	GF/drugs	Clinical	108	ACLR/ST	3 mo–6 mo	Imaging	No difference	2	4/10
2008/Fanton et al ¹⁶	Ketoprofen, amitriptyline, and oxymetazoline	GF/drugs	Clinical	35	ACLR/Allo	1 d–30 d	Clinical, pain	Positive	1	6/10
2008/Benazzo et al ⁴	PEMF	Biophy	Clinical	84	ACLR/HS	30 d–180 d	Quest	Positive	1	4/10
2008/Babb et al ²	MSC	Cell	Immature rabbit	15	ACLR/EXT	3 wk–20 wk	Imaging, histology	Positive	D	5/8
2007/Soon et al ⁵⁵	MSC	Cell	Rabbit	36	ACLR/AT	2 wk–8 wk	Histology, mech	Positive	B	3/8
2007/Li et al ³³	MSC or PDGF-BB-transfected MSC	Cell	Rabbit	36	ACLR/AT	3 wk–12 wk	Histology	Positive	E	1/8
2007/Kanaya et al ²⁷	MSC	Cell	Rat	98	ACLT partial	1 wk–4 wk	Histology, mech	Positive	B	5/8
2007/Huangfu and Zhao ²⁵	TCP	Biomaterial	Dog	48	ACLR/Flex	2 wk–12 wk	Histology, mech	Positive	B	3/8
2007/Huang et al ²⁴	Hyaluronic acid	GF/drugs	Clinical	120	ACLR/BTB	4 wk–16 wk	Clinical, quest	Positive	1	4/10
2006/Yoshikawa et al ⁶⁸	VEGF	GF/drugs	Sheep	18	ACLR/ST	12 wk	Histology, mech, lax	Negative	B	6/8
2006/Dynbil et al ¹³	Osteoprotegerin	GF/drugs	Rabbit	15	ACLR/ST	3 wk	Histology, imaging	Positive	D	6/8
2005/Yamazaki et al ⁶⁶	TGF β 1	GF/drugs	Dog	21	ACLR/Flex	3 wk	Histology, mech	Positive	B	8/8
2005/Ventura et al ⁵⁷	Growth factor	GF/drugs	Clinical	20	ACLR/HS	6 mo	Imaging, quest, biochem	Positive	1	1/10
2005/Kondo et al ³¹	TGF- β , PDGF-BB	GF/drugs	Rabbit	36	Overstretch injury	12 wk	Histology, mech, lax	Positive	A	5/8
2005/Demirag et al ¹¹	Blockade of MMP α -2 macroglobulin	GF/drugs	Rabbit	28	ACLR/ST	2 wk–5 wk	Histot, mech, biochem	Positive	B	3/8
2004/Yasuda et al ⁶⁷	TGF- β + EGF	GF/drugs	Dog	25	ACLR/BTB	12 wk	Histology, mech	Positive	A	5/8
2004/Weiler et al ⁶¹	PDGF-BB	GF/drugs	Sheep	48	ACLR/Flex	3 wk–24 wk	Histology	No difference	A	7/8
2004/Tien et al ⁵⁶	Calcium phosphate cement	Biomaterial	Rabbit	22	ACLR/ST	1 wk–24 wk	Histology, mech	No difference	B	4/8
2004/Lim et al ³⁴	MSC	Cell	Rabbit	48	ACLR/ST	2 wk–8 wk	Histology, mech	Positive	B	4/8
2004/Demirag et al ¹⁰	α -2 macroglobulin	GF/drugs	Rabbit	20	ACLT	10 d	Histology	Positive	E	3/8
2001/Anderson et al ¹	Bone growth factor	GF/drugs	Rabbit	70	ACLR/ST	2 wk–8 wk	Histology, mech	Positive	B	4/8
1997/Kobayashi et al ²⁹	bFGF	GF/drugs	Dog	34	ACLT partial	1 wk–24 wk	Histology	Positive	E	1/8
1997/Kikuchi et al ²⁸	Hyaluronan	GF/drugs	Rabbit	36	ACLT partial	2 wk–6 wk	Histology	Positive	E	4/8
1990/Wiig et al ⁶⁴	Hyaluronic acid	GF/drugs	Rabbit	21	ACLT partial	4 wk–12 wk	Histology	Positive	E	1/8

^aACL, anterior cruciate ligament; ACLR, ACL reconstruction; ACLT, ACL transaction; Allo, allograft; AT, Achilles tendon; bFGF, basic fibroblast growth factor; Biochem, biochemical assay; Biophy, biophysical intervention; BMP, bone morphogenetic protein; BMP2, bone morphogenetic protein-2; BMSC, bone marrow-derived mesenchymal stem cell; BTB, bone-tendon-bone; DB, double-bundle; EGF, epidermal growth factor; EXT, extensor; Flex, flexor tendon; GAS, gastrocnemius; GF, growth factor; HS, hamstring tendon; Lax, laxity test; LIPUS, low-intensity pulsed ultrasound; Mech, mechanical test; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; PDGF-BB, platelet-derived growth factor-BB; PEMF, pulsed electromagnetic fields; PET, polyethylene terephthalate artificial ligament; PRP, platelet-rich plasma; Quest, questionnaire; SIS, small intestine submucosa; ST, semitendinosus; TCP, tricalcium phosphate; TGF- β , transforming growth factor-beta; VEGF, vascular endothelial growth factor.

updated search carried out in January 2014, out of the 331 studies, 199 studies were excluded based on the absence of the keywords *reconstruction*, *reconstructive*, or *healing*, and 22 duplicate studies were removed. After screening the titles of the remaining 110 articles and applying the exclusion criteria based on article type and study type, only 10 studies were included for data extraction.

Data Extraction

The study characteristics of both the original and updated search are provided in Table 2. Of the 60 included studies, 3 tested the effects of biophysical intervention; 11 tested the effects of cells alone or in combination with growth factors

or scaffold, 37 evaluated growth factors or drugs with or without biomaterials, and 9 evaluated the biological effect of biomaterials. Ten clinical studies were included; 2 tested the effects of biophysical intervention and 8 investigated growth factors or drugs. More than half of the animal studies ($n = 31$) utilized a rabbit model, 8 studies used dogs, 3 studies used sheep, 6 studies used rats, 1 study used a porcine, and 1 study used a goat model. Most included studies involved ACLR surgery, except 6 studies that used animal models of ACL partial lesion and 1 on overstretched ACL injury. Various types of grafts were used in the included studies. Both hamstring and bone-tendon-bone (BTB) grafts were used in the clinical studies. In rabbit models, semitendinosus and extensor grafts were preferred but

other graft types were also used. There were no preferred graft types in studies on dogs, and the graft preference was not clear in sheep, goat, or rat models because of the small sample sizes. Two animal studies investigated the effect of biological modulation on allografts, and 2 animal studies evaluated artificial grafts. The maximum follow-up time was 12 months in both clinical and animal studies. Most studies reported positive effects with the biological modulation under investigation; 6 studies reported no difference, and only 2 animal studies reported negative effects with platelet-rich plasma (PRP) and vascular endothelial growth factor (VEGF), respectively. Among the 6 reports of no difference, 3 evaluated platelet factors, 2 biomaterials, and 1 bone marrow concentrate.

Methodological Quality Assessment

The results of the methodological quality assessment are shown in the last 2 columns of Table 2. For clinical studies, 3 were rated as evidence level 2, and the rest were rated as level 1. Four clinical studies scored 6 or higher out of 10 in CASP, while 6 clinical studies scored less than 5 and were considered low quality. For animal studies, 4 studies were ranked "A" regarding clinically relevant quantitative outcome measures, and the methodological scores were all higher than 5 out of 8. Thirty-one animal studies were ranked "B" with the use of mechanical test outcome measures. The methodological score of these studies ranged from 1 to 8, with only 13 studies scoring 5 or higher. Two rank-"C" studies were included with quantitative biochemical outcome measure, and the study quality was low (1 and 3, respectively). Four rank-"D" studies with semiquantitative outcome measures and their methodological score were fair to satisfactory (4 in 1 study, 5 in 2 studies, 6 in 1 study). However, none of the rank-"E" studies scored higher than 4, and all of them were considered low-quality studies. In summary, only 25 included studies were appraised with satisfactory study quality, and further metasynthesis was performed on these studies. Good interobserver reliability was obtained between assessors (S.C.F. and Y.C.C.; intra-class correlation coefficient average measures, 0.848), and consensus on scoring was reached by discussion.

Metasynthesis

In the 25 good-quality studies (methodological score ≥ 5), 1 involved the use of biophysical intervention,¹⁴ 2 used biomaterials,^{23,41} 5 involved the use of cells,^{2,27,30,59,60} and 17 tested the effects of growth factors or drugs.¹¹ Four animal studies found positive results with the use of transforming growth factor-beta (TGF- β) in different combinations to promote bone-tendon junction healing.^{31,48,66,67} Three animal studies tested the effects of VEGF-mediated angiogenesis, but the results were inconsistent.^{6,60,68} Two animal studies tested the effects of platelet-derived growth factor-BB (PDGF-BB) and reported positive findings.^{31,61} The use of bone morphogenetic protein (BMP)-transfected

cells,⁵⁹ osteoprotegerin,¹³ calcium phosphate,^{25,41,51} and demineralized bone matrix²³ primarily targeted osteogenesis inside the bone tunnel, and positive findings were reported. The use of bisphosphonate-targeted inhibition of bone resorption yielded similar effects.^{35,36} One clinical study observed beneficial effects of PRP,⁵⁸ but in another animal study, PRP exerted negative effects in the context of small intestine submucosa (SIS) artificial graft.³² Two animal studies revealed the beneficial effects of supplementation of mesenchymal stem cells (MSCs) alone.^{2,27} Three clinical studies reported improvements in knee functions and pain score with control of postsurgical local inflammation and swelling by electrostimulation,¹⁴ intra-articular injection of autologous serum for interleukin-1 beta (IL-1 β) antagonization,⁹ or drug formulation in arthroscopic irrigation saline for allograft ACLR,¹⁶ whereas 1 animal study reported alleviating inflammation by cationized gelatin and hyaluronic acid coating on artificial graft, which could improve healing at the bone-graft interface.⁸

With respect to outcome measures, 2 clinical studies achieved improved pain and knee functions,^{14,16} while another reported significant improvements in imaging and biochemical outcome measures.⁹ All good-quality animal studies used mechanical tests of pull-out strength of the graft complex as the key outcome except 4 studies, which only reported histological findings alone or together with imaging findings.^{2,8,13,61} One study in a sheep model evaluated healing outcomes with in situ force and knee laxity.⁶⁸ All good-quality studies involved ACLR surgery except 3, which investigated ACL partial lesions in rats^{27,52} or focused on overstretched ACL injury in rabbits.³¹

DISCUSSION

Proposed Mechanism

This systematic review shows that there is good evidence for biological modulation to promote healing in ACLR. The proposed mechanisms for the biological modulation fell into 5 categories: (1) bone-tendon healing at the graft-tunnel interface, (2) angiogenesis, (3) osteogenesis, (4) cell supplementation for general healing capacity, and (5) reduction of local inflammation.

Although the intra-articular midsubstance would be the weakest link of the graft-complex in ACLR, tendon-bone healing at tunnel interface was the target of most biological modulation. Notably, the roles of TGF- β on bone-tendon junction healing have been well demonstrated in other animal models,³⁷ thus it is not surprising to see the effectiveness of TGF- β in promoting graft incorporation in ACLR. Since tunnel widening is an adverse healing outcome of ACLR, strategies that promote osteogenesis or reduce bone resorption also worked to improve the pull-out strength of the graft complex. Studies of cell supplementation also targeted the graft-tunnel interface. However, overall improvement was limited by graft deterioration at the intra-articular space. Strategies to reduce graft deterioration by reducing local inflammation or direct modulation on degradative enzymes^{10,11} may be complementary to

¹¹References 8, 9, 13, 16, 31, 32, 35, 36, 43, 52, 58, 61, 66-69, 73.

treatment that targeted tunnel interface healing. From the search results, only 1 included study¹⁶ used nonsteroidal anti-inflammatory drugs (NSAIDs) as one of the components in the treatment formula. Based on the current search results, it is difficult to conclude that NSAIDs should be used freely for pain control without affecting knee function. However, as inflammation is necessary for the early healing phase and excessive inflammation may hamper the healing outcome, it is critical to control the extent of inflammation. The use of NSAIDs to control excessive inflammation without affecting knee function would be a primary concern for clinicians. With respect to angiogenesis, the current evidence is not consistent, especially with respect to the effects of VEGF. Revascularization would be essential to graft survival, but vascular invasion into the graft may also cause graft weakening. In summary, the current evidence suggests that biological modulation benefited healing by promoting graft incorporation at the tunnel interface, and further works on reducing graft deterioration may be worthwhile.

Practical Significance

For all included animal studies, the pull-out strength of the graft complex in the untreated control group was around 10% of the ultimate strength of the intact ACL, and the improvement by biological modulation was around 22% at maximum. As the clinical graft failure rate for ACLR is around 8%, the practical significance of the improvement in pull-out strength is questionable. However, as ACL in situ force during walking/descending stairs approached 7.8%/20% of ACL ultimate load,⁴⁰ the improvement may help a little to avoid rerupture during daily activities. Knee laxity measurement and knee function scores are routinely used in clinical settings. However, only a few animal studies measured knee laxity and knee functions. Clinical studies showed that treatment delivered intraoperatively or during early healing phases could improve knee function at later time points, indicating the potential of biological modulation of postoperative local inflammation for long-term functional recovery. From the results of the systematic search, we found the maximum follow-up time point is 12 months. Since graft healing after ACL reconstruction is a lengthy process, a longer follow-up of 2 years would better reveal the effectiveness of the treatment modalities.

Representativeness of Animal Models

Currently available clinical studies on biological modulation of ACLR only tested the effects of biophysical intervention, autologous growth factor mixtures, or marketed drugs. The effectiveness of growth factors, cells, and biomaterials was only shown in animal studies, and their validity depends on the representativeness of the animal models. It has been demonstrated that there are significant anatomical differences in ACLs between human and experimental animals,⁴⁷ and the obvious variations in gait and posture defined the differences of the biomechanical properties. However, there is also evidence about the homology of regenerative responses in mammals and the

similar patterns of innervation found in knee joint ligaments.³⁸ Thus, animal studies of ACLR may still convey useful information for further development but extrapolation of preclinical data may need further scrutiny, say, taking a plurispecies approach to confirm the findings.

Factors That Modify Treatment Effects

It is well accepted that evaluation of treatment effects should consider time of application and time of evaluation, dose response, and other ancillary conditions such as rehabilitation and activity levels. As most included animal studies did not include investigation of dose response, the observed treatment effects could not be further confirmed. Moreover, all studies of biological modulation of ACLR used intraoperative delivery except those with biophysical intervention. This is likely because of the practical difficulties of noninvasive delivery of the biological agents during the lengthy postoperative recovery phase. As graft healing in ACLR involves different stages such as inflammation, cell recruitment, revascularization, matrix remodeling for graft incorporation, and ligamentization, there is no doubt that different types of biological augmentation may be needed at different healing stages. Therefore, it is not surprising that positive treatment effects were only observed at early time points but not at later time points as the intraoperative treatment effects would have been faded out.

Analysis of Reporting Biases

This systematic review may be subject to publication bias as most of the included studies reported improvement (52 positive findings, 6 no difference, and 2 negative findings). Only 3 studies with positive results received commercial sponsorship, and their findings did not stand out from similar studies.^{1,4,16} We are certain that there are unpublished studies on biological modulation in ACLR, as shown in the conference abstracts at the Annual Meeting of the Orthopaedic Research Society. Besides, studies in gray literature are difficult to access using the current search strategy. To our knowledge, there are relevant studies published in new journals that are not included in the 3 search databases.¹⁷ Because we identified good-quality studies with an arbitrary cutoff score (≥ 5), it is likely that we may have missed some meaningful findings during the integration of clinical/experimental evidence. If we lowered the cutoff scores to 4, 12 studies would be further included. These studies revealed similar findings according to the mechanisms to promote healing. However, 2 studies reported the positive effects of hyaluronan with repeated intra-articular injection,^{24,28} which may represent a different mechanism for biological modulation of ACLR.

Further Steps to Modify the Current Clinical Practice

Before implementing any biological interventions, it is important to have evidence of their effectiveness for ACLR. Further research is required to determine the necessity and

timing of the interventions. From the clinical studies with reasonably good study quality (score 6-8 out of 10 in CASP), there is evidence that short-term clinical outcomes after ACLR were improved by preparations of growth factors,^{9,58} drugs,¹⁶ or electrostimulation.¹⁴ Most of these interventions improved outcomes related to postsurgical pain response, except 1 study with platelet gel that showed improvement in anterior-posterior knee laxity. These studies suggest that intra-operative interventions targeted to reduce inflammation or promote graft healing are beneficial for recovery after ACLR. Moreover, many preclinical experimental evidences suggested that healing outcome could be further improved with biological modulation. Thus, translational research to bring preclinical findings into good-quality clinical trials is essential for the use of effective biological modulation to improve healing outcomes in a clinical setting. The major trend in ACLR is modification of surgical procedures such as the use of different types of tendon grafts and fixation methods.^{3,19,21} Although this review showed great potential, biological modulations are still in the exploratory stage. More evidence from both preclinical and clinical studies is required for implementation in clinical practice.

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