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Review Article

Augmentation of Bone Tunnel Healing in Anterior Cruciate Ligament Grafts: Application of Calcium Phosphates and Other Materials

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Bone tunnel healing is an important consideration after anterior cruciate ligament (ACL) replacement surgery. Recently, a variety of materials have been proposed for improving this healing process, including autologous bone tissue, cells, artificial proteins, and calcium salts. Amongst these materials are calcium phosphates (CaPs), which are known for their biocompatibility and are widely commercially available. As with the majority of the materials investigated, CaPs have been shown to advance the healing of bone tunnel tissue in animal studies. Mechanical testing shows fixation strengths to be improved, particularly by the application of CaP-based cement in the bone tunnel. Significantly, CaP-based cements have been shown to produce improvements comparable to those induced by potentially more complex treatments such as biologics (including fibronectin and chitin) and cultured cells. Further investigation of CaP-based treatment in the bone tunnels during ACL replacement is therefore warranted in order to establish what improvements in healing and resulting clinical benefits may be achieved through its application.

1. Introduction

In all types of anterior cruciate ligament (ACL) reconstruction, a proportion of grafts fail due to a lack of healing in the bone tunnel or abrasion of the graft at the tunnel exit [1]. It has been suggested that stable bone tunnel healing is desirable for an ACL graft to be successful [2] and that the acceleration of the healing between a soft tissue (tendon) graft and bone may allow earlier return to functional activities and improve clinical outcomes [3]. Unsatisfactory osseointegration of tendon grafts used for the replacement of ACL may also be associated with postoperative anterior-posterior laxity [4]. It may therefore be expected that improvements in bone tunnel healing, including ingrowth or ongrowth of tissue around the graft, will improve fixation strength and limit graft failures by pullout and loosening.

A variety of materials have been applied in the bone tunnels in order to improve healing. These range from autologous bone tissue or cells to proteins and calcium salts. In particular, a number of studies have proposed the use of calcium phosphate (CaP) materials for this purpose. The application of CaP to soft tissue attachments is becoming more common and has been shown to induce increases in fixation strengths and bone formation.

This review examines the hypothesis that the application of CaP can improve bone tunnel fixation and healing in ACL grafts. The evidence for the usefulness of CaPs in ACL replacement is discussed along with evidence for the efficacy of CaP in other relevant applications and other materials used for bone tunnel healing improvement. The following section gives a brief introduction to ACL replacement, mechanisms of bone tunnel healing, and methods commonly used to assess healing.

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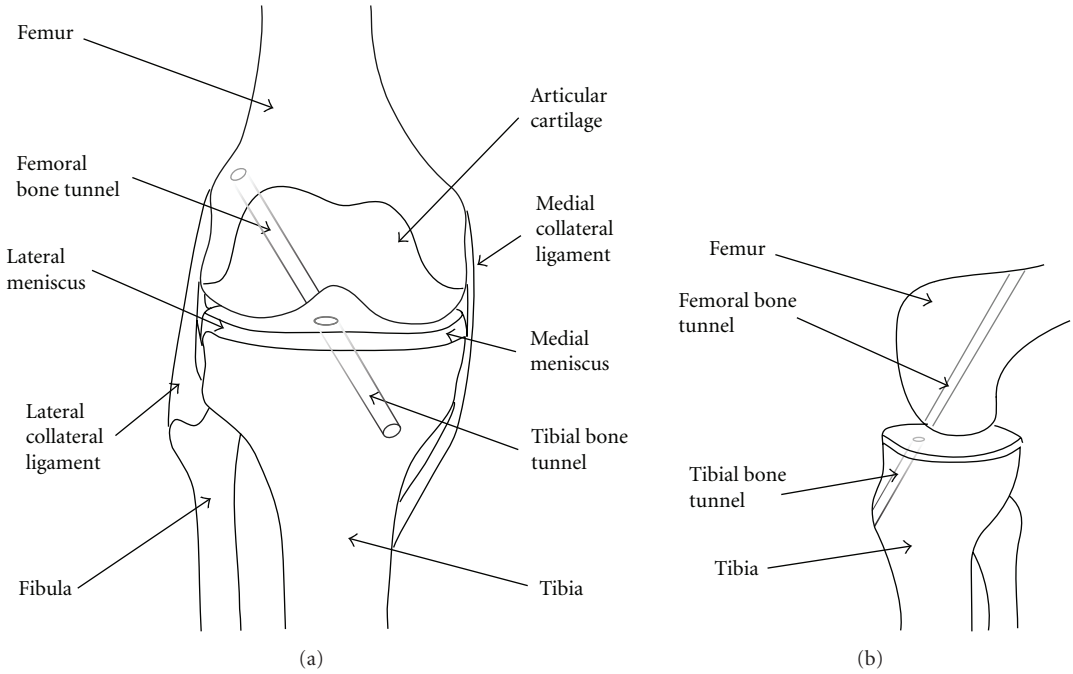


Figure 1: The anatomy of the bones of the knee showing the position of the bone tunnels from (a) frontal and (b) lateral views. The patella and patellar tendons are omitted for clarity.

2. Bone Tunnel Healing in ACL Replacement

2.1. ACL Anatomy and Injury. The ACL is one of two ligaments located in the centre of the knee joint. It has insertions in the tibia and distal femur and has a microstructure made up of collagen bundles surrounded by a complex matrix [5]. The ACL restricts anterior tibial translation and gives stability in rotation. Failure to treat this type of injury may lead to mechanical instability in the knee and has been linked to the early onset of osteoarthrosis [6]. Damage to the ACL may occur from both contact and noncontact mechanisms of injury [7]. It is common both in the general population and, more particularly, in those taking part in sporting activities. A recent study based in the United Kingdom identified the rate of ACL injury at 8.1 instances per 100,000 people per year [8]. Reconstructive surgery is routinely carried out for patients with a torn or ruptured ACL. In the USA, around 200,000 ACL reconstructions are performed each year [9]. The reader is referred to recent reviews for detailed information on ACL anatomy, function, and injury [5, 10, 11].

2.2. ACL Replacement. The ACL does not heal when torn because it lacks sufficient vascularisation. Surgical reconstruction is the standard treatment in sports medicine for ACL rupture [5]. Patients with ACL injuries are typically younger and more active than other orthopaedic patients, and reconstructions should exhibit good longevity, withstanding high stresses over millions of cycles [12]. However, the outcomes of the various techniques used for ACL replacement have not always been positive. Patient selection and implantation technique have contributed to poor results from artificial grafts, allografts, and autografts.

Over the past 10 years, technique has vastly improved, making autograft ACL replacement both common and more successful. The procedure is usually carried out arthroscopically using a graft from the semitendinosus tendon or the central third of the patellar tendon. In the latter case, the graft is harvested with a section of bone at each end and interference screws are used to fix the bone plug into place. These are known as bone-tendon-bone (BTB) grafts. A recent review suggests that there is no significant difference in clinical results between autograft types, with factors other than graft donor site (including fixation, damage to the meniscus and articular cartilage, and the requirement of additional surgical procedures) being the most important determinants for successful outcomes [13].

Once the graft has been harvested and prepared, often by pretensioning and the addition of sutures, tunnels are drilled through the tibia and femur passing through the attachments of the original ligament (see Figure 1). The graft is pulled into position and fixed using staples, screws, sutures, or commercially available fixation devices such as the cross-pins or interference screws. The surgical fixation is the weak point of the graft in the early postoperative stages and remains so until bone tunnel healing occurs [14].

Autografts for ACL replacement have limitations including limited availability, and adverse functional changes including muscle weakness at the donor site. Conversely, artificial or tissue-engineered ligament grafts have some distinct advantages. These include the ability to control manufacturing, condition, quality, sterility and size of device before implantation. Mechanically tested and controlled grafts could be made available off the shelf and eliminate the need to create a second defect site through the harvesting of

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healthy tissue. Unfortunately, the majority of artificial ACLs have suffered from high failure rates due to mechanical, and in some cases biological, influences and have been removed from the market [12]. In the early postoperative stages, the majority of these failures occurred in the bone tunnels.

2.3. Bone Tunnel Healing. The insertion of the native ACL is characterised in four layers: tendon, fibrocartilage, mineralised fibrocartilage, and bone. The collagen fibres of the tendon extend into both the fibrocartilage, and the mineralised layer. This structure is usually destroyed when the ligament is removed and the bone tunnel is drilled. A replication of this direct type of insertion may be considered desirable when assessing bone tunnel healing for ACL grafts.

The mechanism by which graft-bone healing occurs depends on the type of graft used. For BTB grafts, healing in the tunnel resembles normal fracture healing, but may be a more complex process. Incorporation of the bone block in the tunnel has been observed as early as 16 weeks after surgery [15]. BTB grafts have the advantage of allowing rigid fixation of the graft in the bone tunnel.

The tendon-bone healing process occurs through a different mechanism after implantation of a soft tissue graft without bone plugs [15]. Firstly, fibrovascular tissue forms between the graft and bone and becomes mineralised. The tendon tissue itself is then mineralised and incorporated into the bone [16]. Sharpey's fibres are made up of type I collagen and connect the periosteum to the bone. The formation of Sharpey-like fibres within the bone tunnel is often identified as a marker of indirect healing between the tendon and bone [17]. The formation of these collagenous fibres may occur from six weeks after surgery. However, complete bone tunnel healing of an ACL graft may occur as late as six to twelve months after surgery [15]. Some studies in animals have suggested that tendon graft incorporation occurs more slowly than BTB healing [2].

In addition to the choice of graft, surgical fixation, and graft position, interfacial motion within the bone tunnel may affect healing [2]. Graft motion within the bone tunnel has been shown to be inversely proportional to healing in an animal model [18].

2.4. Assessment Techniques for Bone Tunnel Healing. Three main factors are commonly assessed to evaluate bone tunnel healing after ACL replacement: functional outcome, biological structures, and mechanical properties. In clinical studies, functional outcome is measured by patient satisfaction, pain levels, and scores in the International Knee Documentation Committee (IKDC) and Knee injury and Osteoarthritis Outcome Score (KOOS) tests. In both clinical and animal studies, the biological structures in the bone tunnel are examined using noninvasive imaging. In animal models, these structures can also be examined by the excision and histological examination of the graft-bone construct. This method can be used to identify collagen fibres, calcified tissue and different types of cell found in the bone tunnel. Imaging techniques include the use of X-rays for the assessment of bone tunnel width and, more recently, the use of CT scanning to quantify the amount of bone tissue formed within the tunnels.

Biomechanical tests are carried out on the tibia-graft-femur construct following *in vivo* studies in animals. This testing yields strength and stiffness data, as well as permitting observation of the mode of failure of the graft. The explanted bone-graft construct is mounted in a tensile test machine and, most commonly, extended to failure at a constant rate. The ultimate tensile strength (UTS) is recorded, and graft stiffness may be calculated. Direct comparison of data from these tests is complicated by differences in angles of flexion chosen when mounting the bones for the test as well as variations in extension rate, pretensioning or cyclical loading of the graft and the type of animal model used. In particular, the rate of extension may influence the mechanical behaviour of the graft [25].

Two distinct modes of failure are reported in tensile testing. Pullout is the term commonly used to indicate a failure of the fixation or tendon-bone interface. The pullout strength of a graft is the force required for failure to occur by this mode. The UTS of a graft may refer to pullout or to midsubstance failure, which describes the rupture of the graft material itself or, in some cases, the deformation of the graft beyond a functional length. The majority of studies combine load-to-failure data, irrespective of the mode of failure which occurred. It is expected that failures in the early postoperative stages will occur in the bone tunnel, with midsubstance failure becoming the more common mode as bone tunnel healing advances, leaving the soft tissue graft itself as the weakest point in the construct [14]. A difference in the mode of failure occurring in experimental and control groups may therefore be interpreted as an indication of an increase in strength of the bone-graft interface, assuming the bone healing enhancement does not weaken the graft.

3. Calcium Phosphates

3.1. Calcium Phosphates as Biomaterials. CaPs are considered to be safe, biocompatible materials for use in long-term implantation. They have been used in a variety of applications including hip stem coatings and bone graft materials and are commercially available in injectable, powder, granular, and block forms.

CaP is bioactive: the presence of Ca and P ions allows the formation of a direct chemical bond between the bone and the implant [26]. The exact properties of CaPs depend on the Ca : P ratio, the crystallinity of the material, the presence of water and the purity of the material [27]. Hydroxyapatite (HA) has Ca : P ratio of 1.67 and may be considered as stoichiometric CaP. CaP occurs naturally in the body as the mineral component of bone and enamel in a form resembling HA [28]. β-tricalcium phosphate (β-TCP) is a more quickly resorbed form of CaP. HA is resorbed over a period of decades, while β-TCP resorbs in months [29]. The adsorption of particles on the surface of CaP is related to its crystallinity and influences the biological response to the material. CaP powders of different sizes have been shown to produce differing rates of bone formation *in vivo* [30]. The reader is referred to a review of bioceramics for more information on general uses and properties of CaP [31].

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Table 1: Mechanical testing of different augmentation methods using calcium phosphates for bone tunnel healing. For ease of comparison and where available, data from mechanical testing at six weeks after surgery are given. Results at earlier and later time points are detailed in the text. Where six week data is not available, the nearest time period is included. [a]These values were calculated from the average values in the preceding two columns. [b]Reflects a difference in the predominant mode of failure from pullout failure in the controls to midsubstance failure in the treated subjects. [c]This is a low-porosity CaP control, not a nontreated sample.

Augmentation method	Average increase in strength (as % of control)[a]	Ultimate tensile strength in treated group	Ultimate tensile strength in control group	Change in mode of failure[b]	Time (weeks)	Reference
Brushite calcium phosphate cement (CPC)	118%	94 ± 42 N	43 ± 11 N	✓	6	[19]
Injectable tricalcium phosphate cement (TCP)	87%	62.90 ± 7.62 N	33.60 ± 5.87 N	✓	4	[20]
Injectable CaP cement	110%	11.491 ± 2.865 N	5.253 ± 3.955 N	×	2	[21]
Injectable CaP with magnesium	65%	71.8 ± 31.8 N	43.4 ± 14.8 N	×	6	[22]
Hybridization by CaP precipitation	Not statistically significant	116.9 ± 48.3 N	109.4 ± 47.2 N	2/7 failed by pullout in treated group, 3/7 in control	6	[23]
Bulk CaP with interconnected pores	116%	12.8 ± 5.9 N	5.0 ± 1.8 N[c]	×	6	[24]

In this review, CaP is employed as a general term for calcium phosphate-based materials and is used where the specific type of CaP is not identified in the original study. The specific type of CaP produced or used in a study is identified when possible.

The following section is a review of studies using CaP to improve bone tunnel healing. Both qualitative and quantitative assessments are considered, along with analysis of biomechanical changes induced by the treatments. A summary of the outcomes of mechanical testing from these studies is given in Table 1.

3.2. Calcium Phosphates in ACL Reconstruction

3.2.1. Injectable Materials. A short-term biomechanical study on the effects of CaP cement on the pullout strengths of tendon grafts for ACL replacement was carried out using a rabbit model by Tien et al. [21]. Grafts were implanted bilaterally and held in place with sutures. One graft in each subject was then further fixed in place by injection of the bone cement into the tunnel. The application of the cement led to increase in pullout strength, with the average strength more than doubled as measured two weeks after surgery. Biomechanical testing was not carried out at later time points. Histological examination showed bone islands growing between the cement, bone and tendon as early as three weeks after implantation. Bone development extended to 24 weeks after surgery. In the noncemented control subjects, no bone formation was found in the interfacial gap.

These findings are supported by Huangfu and Zhao, who examined the use of injectable resorbable TCP in bone tunnels in a canine model [20]. The grafts filled only the articular ends of the tunnels and were fixed using sutures. The CaP was then used to fill the sections of the tunnels not filled with the graft. Although the TCP was not specifically injected into the tendon-bone interface, it was observed to be present in that area during histological evaluations. At 12 weeks after implantation, in the experimental grafts, areas resembling a normal ACL insertion appearance with fibrocartilage and calcified bone were present. The remainder of the interface showed a regular Sharpey-like fibre link from tendon to bone. Bone development in the controls was found to be slower, with no calcified tissue or fibrocartilage formed. The pullout strength of the grafts was found to be increased by the presence of the TCP up to six weeks after surgery. While earlier results were statistically significant, the significance of data gathered at the six week time point was limited by the size of the sample. From eight weeks after surgery, all failures in the test group occurred by mid-substance rupture. This study established a clear pattern of improved intratunnel healing up to 12-weeks after surgery.

The authors of these studies do not discuss whether the observed improvement in healing was the result of the restriction of graft movement in the tunnel due to the presence of the cement or of increased bioactivity due to the chemical effect of the CaP. Huangfu and Zhao do, however, speculate that the use of a resorbable material is preferable if a normal ligament-bone insertion is to be developed [20].

In a further study examining the effects of CaP-based cements, a cement containing brushite (dicalcium phosphate dihydrate) was shown to increase fixation strengths in ACL grafts up to 12 weeks after implantation in a rabbit model [32]. The increase in strength was 118% six weeks after surgery and 55% at the 12 week time point. The cement was injected into the bone tunnels before the grafts were pulled into place. *In vivo*, the cement degraded to leave granules of β tricalcium phosphate between the bone and tendon. The majority of failures in the treated group occurred in the intra-articular section of the graft, whereas control grafts failed by pullout. The increase in strength corresponded

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with larger amounts of bone formation around the tendon graft.

Gulotta et al. investigated the use of an alternative to standard injectable CaP materials by adding magnesium to the cement [22, 33]. While standard CaP cements act as grout, filling the space between the bone cavity and the graft, the inclusion of magnesium was intended to give this product adhesive properties. The study was carried out in a rabbit model, and grafts were held in place using sutures. In the control group, no cement or adhesive was applied. Three weeks after surgery, the strength of the experimental group was the same as that of the controls. However, this may have resulted from incomplete hardening of the adhesive at this stage. Six weeks after surgery failure loads were 65% higher than the controls. The failure of the grafts is described as occurring at the "graft-tunnel junction." The authors note that although the graft fixation strength was increased in this study, it did not achieve the strength of an unoperated tendon. The average ultimate load-to-failure of the native rabbit ACL has been shown in a previous study to be 351.8 ± 41.6 N (Labs 2002 cited by [22]), while mean load to failure in the treated group was 71.7 N [22]. This study also made use of μCT scanning to quantitatively measure the increase in bone volume in the tunnels. A significant increase in total intratunnel bone volume was observed in the experimental group when compared to control at six weeks. Staining also showed more cartilage tissue and less fibrous tissue formation in the bone tunnels. The increase in cartilage formation was shown to be statistically significant at six weeks after surgery, as evidenced by an increased area of metachromasia ($79\,556.2 \pm 61\,664.0\,\mu m^2$ compared with $2806.2 \pm 6\,873.7\,\mu m^2$ for the control) [22].

Although the results of this study show improvement when compared to controls in which no bone adhesive was used, the role of the magnesium in this improvement has not been proven. When comparing the results of this study to others using CaP cements, there does not appear to be an increase in strength corresponding to the presence of the magnesium (see Table 1).

It is important to note that the use of bone cements in ACL graft attachment without additional surgical fixation has been shown to result in inadequate fixation [34]. An *in vitro* study in porcine bone compared various methods of surgical fixation, including a calcium carbonate-containing cement. High levels of graft slippage within the bone tunnels were observed during cyclical loading, showing the bone cement to be unsuitable as a primary fixation method.

A different injectable material was proposed by Ishikawa et al. [35]. Collagen gels containing HA for the improvement of tendon-bone healing were tested in a rabbit model. A direct bond was shown to be formed between the tendon and the bone in the presence of the gel, which contained 60% HA and 40% collagen. The HA particles were up to 200 μm in size. The presence of both the collagen and the HA resulted in collagen fibres from the tendon being interwoven into newly formed bone around the graft. In the controls, in which no gel was applied, amorphous tissue formed in the tendon-bone interface. The effect of the improved interface on the mechanical performance of the graft was not assessed.

3.2.2. Bone Screws in ACL Reconstruction. A further means of introducing CaP into the bone tunnel is to include them in the material to be used in the fixation of the graft. This commonly involves the use of resorbable fixation screws containing CaP. A number of studies have examined the use of CaP-containing interference screws for soft tissue graft fixation. Hunt et al. compared bone tunnel healing for grafts fixed with commercially available PLLA-HA composite screws with that for grafts fixed with simple PLLA screws in ovine models over a period of 12 months [36]. New bone formation along the perimeter of the screw threads was found to be significantly increased in screws containing HA than those containing PLLA alone. These observed increases in bone ingrowth and mineralisation can be directly attributed to the presence of the HA as the mechanical fixation of the two types of screw is comparable. The mechanical properties of the fixations and the phenomenon of bone tunnel widening were not investigated.

The same composite screws (HA/PLLA) have also been examined *in vivo* in a clinical setting in 100 patients [37]. The results supported those of Hunt et al., with a reduction in tibial tunnel widening occurring around the screw in cases where the composite screws were used. It is interesting to note, however, that above the screw, in the section of the tunnel containing tendon graft, bone tunnel widening was unaffected by the type of screw used. This suggests that the effect of the HA is highly localised. The improvement in bone tunnel healing around the screw did not correspond to any difference in clinical outcome or knee laxity. However, this study was carried out 12 months after surgery. More differences between the experimental and control groups may become apparent at later time points.

3.2.3. Precipitation of CaP. In contrast with other methods which seek to apply CaP in the bone tunnel or include it in the fixation, Mutsuzaki et al. deposited a layer of CaP directly onto a tendon graft [23, 38]. This was achieved by soaking the ends of the tendon in Ca-containing solution and a PO4-containing solution in turns for 30 seconds each. The complete soaking process took ten minutes, and the CaP layer deposited was over 100 μm thick. XRD analysis showed the deposited material to be made up of low-crystallinity apatite and dicalcium phosphate dihydrate. The deposited CaP was examined by transmission electron microscopy and was shown to be made up of needle-like crystals formed on and between the collagen fibrils of the tendon [23].

When implanted in white rabbits, the "hybridized" tendons appeared to heal faster than controls which had been soaked only in saline. As early as 5 days after surgery, increased numbers of osteoclasts and osteoblasts were observed in the experimental tendons compared to the controls. Over a period of four weeks, tendon-bone healing was more advanced in the healing group, particularly in the formation of a direct tendon-bone bond, without the layer of interfacial fibrous tissue observed in the controls [38]. Although the later study implanting hybridized tendons in goats failed to find a corresponding increase in UTS six weeks after surgery, a slight change in the failure mode was observed between experimental grafts and controls [23]. Failures in

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the CaP treated grafts occurred in the intra-articular portion, whereas three of the seven control grafts failed by pullout from the bone tunnel. The authors claim that this implies that the fixation in the CaP grafts is stronger than that in the controls and may be related to the earlier observation of improved bone tunnel healing; however, the assessment is not statistically significant. The studies were carried out six weeks after implantation.

In addition to studies directly investigating bone tunnel healing, CaPs have been used for enhancement of the attachment of other soft tissues grafts to bone. The following section presents the outcomes of investigations into these applications of CaP-based materials.

3.2.4. Calcium Phosphates in Other Relevant Applications. The use of porous CaP blocks has been suggested as a means of attaching tendon to bone. Although their study was not based on an ACL replacement procedure, Omae et al. examined healing between two types of porous CaP and tendon grafts implanted with them in rabbit femora [24]. The two materials tested were both commercially available in Japan. The first had a pore size around 150 μm, was 72%–78% porous and was made up predominantly of interconnected pores. The second material had a pore size of 50–300 μm and 35%–48% porosity with a lower level of pore interconnection. Wedges or cylinders of the CaP were implanted in the bone with cylindrical holes allowing the tendon graft to be passed through the block. The material with the interconnected porosity induced the best healing, with early formation of collagenous tissue followed by bone ingrowth into the material. Twenty-four weeks after surgery the tendon was found to be in direct contact with the bone grown into the CaP material. The amount of biological ingrowth into the other material was found to be lower. This was a predictable outcome due to the lack of interconnectivity in the porous material. The improved ingrowth in the interconnected material resulted in an increase in tendon pullout strength. This paper does not, however, comment extensively on the healing between the CaP and the tendon, focussing instead on the extent of bone ingrowth into the porous material. An extension to this study found that seeding bone marrow stromal cells into the interconnected CaP ceramics further improved bone attachment [39]. This procedure could be considered a step towards replicating a BTB graft by artificial means.

CaP is also used as a synthetic bone graft and has been proposed for use in a variety of forms as a scaffold component for tissue engineering. Some of these applications may be transferable to ACL graft fixation, particularly in the development of artificial grafts. The following brief review summarises investigations of CaP which may be relevant or applicable to ACL graft development.

Al Munajjed and O'Brien produced collagen scaffolds and coated them in precipitated hydroxyapatite by serial soaking in calcium chloride and ammonium sodium hydrogen phosphate solutions [46]. The scaffolds produced were not sufficiently strong for implantation in bone without support, having a compressive modulus of 10.3 KPa. However, this material combination may have applications in bone tunnels, particularly if the collagen-CaP structure could be tailored to encourage regrowth of a gradual structure mimicking the natural ACL insertion.

Mavis and Demirtas used a simulated body fluid-like solution to deposit nanoscale HA particles on polycaprolactone nanofibres. The aggregation of the HA did not compromise the porosity of the resulting scaffold. The presence of HA was shown to increase the attachment and proliferation of osteoblast-like (MC3T3) cells on the scaffolds *in vitro* [47]. Other HA-containing polymer composites proposed for bone tissue engineering include HA-Poly(ester urethane), which was shown to retain its viscoelastic properties and biocompatibility after HA incorporation [48] and HA-polyamide [49].

The development of synthetic materials for the replacement of articular cartilage has advanced in recent years. The production of compliant materials which mimic more closely the properties of natural cartilage necessitates the development of a means of fixing the graft to the underlying bone. As suggested by Sinha and Guha, the incorporation of HA into an appropriate scaffold material may facilitate fixation to bone tissue [50]. In this study, HA-PVA hydrogels were obtained via the freeze-thawing of a PVA emulsion in which HA particles had been made to precipitate. The resulting scaffolds were porous and the authors suggest that it may be possible to induce a gradient in the HA concentration through the structure, making it suitable for bone-cartilage tissue engineering [50]. Similarly, Wu et al. investigated a PVA hydrogel for cartilage replacement [51]. The HA particles were found to increase elastic modulus of the material. *In vitro*, the presence of HA also increased apatite formation when submerged in simulated body fluid. This is often interpreted as a sign of bioactivity and is a commonly observed phenomenon in HA-containing materials [52].

These promising *in vitro* indications are complemented by a further study which included an *in vivo evaluation*. An HA-PVA hydrogel construct with a graduated HA content was fabricated by a sol-gel method by Zheng et al. and tested both *in vitro* and *in vivo* [53]. PVA does not usually adhere to cartilage and living bone. After immersion in SBF, only HA-containing materials were coated in a biomineralised CaP layer. This corresponded to good bonding and osteoid development between the subchondral bone and the synthetic material when implanted in the femoral heads of rabbits. The authors considered HA-PVA to be a promising articular cartilage construct, particularly with respect to its bone integration.

The addition of HA to PVA in order to improve cell attachment properties was also put forward by Degirmenbasi et al. [54] for use in articular cartilage replacement. The HA/PVA/collagen scaffolds produced were porous, a feature desirable for the encouragement of bone ingrowth. However, the pores produced measured no more than 500 nm, a dimension too small to allow bone ingrowth to occur [55].

An alternative approach to improving PVA attachment to bone in cartilage repair is to coat the hydrogel attachment surface with a layer of amorphous HA to provide an interface. One study coated the bone-contacting surfaces of a PVA hydrogel construct with amorphous HA using pulsed laser

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Table 2: Mechanical testing of various augmentation methods for bone tunnel healing. For ease of comparison and where available, data from mechanical testing at six weeks after surgery are given. Results at earlier and later time points are detailed in the text. Where six week data is not available, the nearest time period is included. [a]These values were calculated from the average values in the preceding two columns. [b]Reflects a difference in the predominant mode of failure from pullout failure in the controls to midsubstance failure in the treated subjects.

Augmentation method	Average increase in strength (as % of control)[a]	Ultimate tensile strength in treated group (N)	Ultimate tensile strength in control group (N)	Change in mode of failure[b]	Time (weeks)	Reference
GCSF	114%	99.45 ± 25.5	31.97 ± 11.9	✓	4	[40]
BMP-2 (low dose)	0%	142 ± 50	143 ± 68	✓	4	[41]
BMP-2 (high dose)	Not statistically significant	210 ± 66	171 ± 20	✓	4	[41]
BMP-7	77%	380 ± 33	215 ± 44	✓	6	[42]
Xenograft-derived BMP	52%	64.71 ± 21.36	42.69 ± 15.03	×	6	[43]
Stem cells	122%	55.7 (Range 21–90)	30.6 (Range 18–43)	×	4	[44]
Periosteum	43%	46.9 ± 13.3 N/mm	32.7 ± 13.3 N/mm	✓	6	[3]
Periosteum	77%	57.1 ± 16.7	32.23 ± 9.9	2/10 failed by pullout in treated group, 1/10 in control	6	[45]
Periosteum with bone marrow	Not statistically significant	35.39 ± 9.3	32.23 ± 9.9	3/10 failed by pullout in treated group, 1/10 in control	6	[45]

deposition (PLD) [56]. This technique has the advantage of allowing targeted deposition which, unlike soaking methods, leaves the articular surface of the PVA clear of HA. When tested *in vitro*, the presence of the 300 nm thick layer of HA greatly increased the attachment and proliferation of murine fibroblasts (L929). The investigation was continued with a study of osteoblast cell (MC3T3) attachment to HA-covered gels [57]. Cell numbers were higher on the HA than on the hydrogels alone, as were both alkaline phosphatase and osteocalcin production. The presence of the HA encouraged osteoblast differentiation. The authors consider this an indication that HA coating by PLD is an effective way of fixing PVA hydrogels to bone.

A recent review considered the range of materials applied for the enhancement of intra-tunnel healing [2]. The following section briefly presents these strategies for the augmentation ACL graft incorporation before comparing their results with those found for CaPs. Table 2 summarises the effects on fixation strengths documented for some of the different methods.

4. Alternative Augmentation Materials

4.1. Soft Tissue Grafts

4.1.1. Biologics. The use of artificial or processed bone proteins and growth factors to augment healing in ACL replacement grafts has been investigated. Granulocyte colony stimulating factor (GCSF) causes the production of granulocytes and stem cells in bone marrow. It has been shown to induce the differentiation of neutrophils (cells of the immune system associated with inflammation) and to encourage angiogenesis and the differentiation and migration of mesenchymal stem cells. Sasaki et al. therefore proposed that the application of GCSF may encourage accelerated bone tunnel healing. GCSF was incorporated into a gelatin hydrogel to control its release and applied during ACL reconstruction in adult beagle dogs. In biomechanical tests, the treatment resulted in a large increase in the failure load (see Table 2). Histological investigations also indicated accelerated bone development around the GCSF treated grafts [40]. The majority of experimental grafts failed midsubstance while untreated grafts failed by pullout, indicating an increase in the bone-graft interface.

Bone morphogenetic proteins (BMPs) are signalling proteins that influence tissue structures in the body. They have been shown to have a role in skeletal development. Both BMP-2 [58] and BMP-7 [42] have been shown to increase graft fixation strengths when applied in the bone tunnels in animal models. Likewise, BMP-7 was shown to increase the volume of bone formed within the tunnels six weeks after implantation [42]. However, one study found that the difference in strength between grafts treated with BMP-2 and controls diminished over time (as measured eight weeks after surgery) [41]. This implies that these products induce faster healing, but not necessarily stronger fixations in the long-term.

Chen et al. advanced the study of the use of BMPs by combining them with implanted periosteal progenitor cells [4]. The protein was tested in soluble form (BMP-2 alone) and tethered to the surface to prevent dissipation (BMP-2 tethered with hyaluronic acid). At three and six weeks after surgery, more calcium and collagen were found in the soluble BMP-2-containing samples than in controls, with significantly increased amounts identified in the hyaluronic-acid tethered samples. At three weeks after surgery, the mode of failure was changed in the hyaluronic-tethered BMP-2 group, in which no samples failed by tibial pullout.

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Failure strengths were higher in the treated groups than in the control, with the hyaluronic-acid tethered grafts the strongest six weeks after surgery. Similar results to those found for BMPs were observed after application of a bone-derived extract (Bone Protein, Sulzer Orthopaedics) [59]. In tensile tests, failure loads were significantly higher than in control groups at two, four and eight weeks after surgery, although the failure modes were unchanged.

Bone samples can be used as a source of natural BMPs. Pan et al. studied the effect of applying recombined bone xenograft within the bone tunnels after ACL replacement [43]. The xenograft was used to produce BMPs, which were then mixed with cancellous bone and formed into cylinders which were attached to the ends of tendon grafts and implanted in the tibial and femoral bone tunnels in rabbit models. The average load to failure of the treated grafts at this time point was 58% greater than that of the controls. Failure strength at 12 weeks after surgery was also increased.

Demineralised bone matrix (DBM) is a further source of BMPs which has been proposed as a means of enhancing tendon-bone healing in rotator cuff repair. Application of this material has been shown to increase fixation strengths between tendon and bone in an ovine patellar model. The presence of the DBM induced an increase in the growth of fibrocartilage and mineralised fibrocartilage at the tendon-bone interface [60].

These studies demonstrate the importance of the mode of delivery chosen for these proteins, as shown by the differences in results between tethered and nontethered molecules. Longer studies would be beneficial in order to properly evaluate their efficacy. BMPs have been applied in other bone repair applications, producing promising results, and BMP-containing products have been approved by regulatory bodies. The reader is referred to a recent review for further information [61].

4.1.2. Cells. As well as the use of biologics (which include BMPs and GCSF), the application of materials seeded with mesenchymal stem cells (MSCs), from which osteoblasts are derived, has also been shown to increase failure strengths and improve bone tunnel healing. In one such study, MSCs were applied to the surface of a graft, seeded in a fibrin glue carrier. The difference in strength between the experimental and control groups was shown to increase over time after application of these cells up to eight weeks after surgery. This trend is in contrast with those observed for other interface healing enhancement materials. Histological examination showed the presence of type II collagen at the tendon-bone interface eight weeks after surgery. Histological characteristics of the interface were found to be similar to normal rabbit ACL insertions [44]. The development of insertion architecture comparable to that of the native ACL is desirable if it results in comparable strengths and loading responses in the graft.

4.1.3. Periosteum. The augmentation of intra-tunnel sections using periosteum has been proposed due to the osteogenic potential of periosteal cells and tissue. A number of studies [2, 62, 63] have found improvements in intra-tunnel bone development and an increase in mean load to failure using this technique. A further study found that while the periosteum treated grafts displayed higher strength than the control grafts treated six weeks after surgery (see Table 2), the difference at 12 weeks was not statistically significant. An additional group of grafts in this study were treated with bone marrow in addition to periosteum. No statistically significant difference was observed in fixation strength six weeks after surgery. However, at 12 weeks an increase of 47% was observed [45].

The harvesting process for periosteal tissue is fast, requiring only three additional minutes of surgery [62]. In animal models, the addition periosteal tissue to tendon grafts provided increased strength and resulted in a change of failure mode compared to controls [3]. This technique has the advantage of delivering autologous bone-forming cells to the bone tunnel. It is also possible that the presence of the layer of periosteal tissue in the tunnel provides some mechanical benefit in limiting the movement of the graft within the tunnel.

Tendon grafts remain the "gold standard" in ACL replacement, despite various artificial grafts which have been proposed over the last thirty years. Although ultimately these artificial grafts were not considered successful, useful information may still be obtained from attempts to encourage long term fixation by improving bone healing around them. The following section reviews additional means for improving graft fixation that has been employed when implanting artificial ACL grafts.

4.2. Artificial Grafts. Prosthetic grafts for ACL replacement have been available since the 1970s. Examples of the materials used for these devices include carbon, Gore-Tex, Dacron, polypropylene, and polyethylene terephthalate (PET). Generally, outcomes of these devices have been poor, leading to the withdrawal from the market of the vast majority of artificial grafts. Failure modes for artificial grafts have included intra-articular rupture, foreign body reactions and loosening, abrasion at the bone tunnel exit, failure of fixation and poor intratunnel healing [12]. Abrasion at the bone tunnel exit has been shown to be significantly reduced by chamfering of the corners of the bone around the tunnel [1]. A number of studies have considered the promotion of intra-tunnel healing in artificial grafts. For example, the Leeds-Keio ligament, a polyester mesh intended to act as a scaffold for soft tissue repair, was fixed in place using bone plugs, replicating the BTB type autografts [12].

Following disappointing outcomes for artificial grafts due to wear and abrasion, the inclusion of biological components to allow for graft remodelling has been the subject of several papers. In the native ACL, the primary zones of the natural ligament-bone insertion structure (ligament, fibrocartilage, mineralised fibrocartilage, and bone) are populated by different cell types. Spalazzi et al. recently developed a three-phase scaffold designed to mimic this interface [64]. This single structure was made up of a poly(lactic-co-glycolic acid) (PLGA) mesh for fibroblast and soft tissue culture, PLGA microspheres for the transition

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zone and sintered PLGA and bioactive glass for the bone section. The device is suggested for use as a graft collar in ligament grafts. It has been shown to support the growth of multiple cell types (seeded *in vitro*) when implanted subcutaneously in an animal model but is yet to be tested functionally.

Chitin is a biopolymer found in the exoskeletons of crustaceans and insects. It is considered to be a bioactive material. One study proposed the application of chitin as a means of improving the attachment of artificial ligaments. This *in vivo* study in a rat model showed that the application of chitin/chitosan to a polyester fabric significantly increases pullout strength and bone formation in the short term [65]. The pullout strength of the treated samples was found to be twice that of the nontreated polyester controls two weeks after implantation. The advanced bone growth is attributed by the authors to the bioactivity of chitosan, including its ability to promote osteoblast attachment and extracellular matrix production. However, images of the coated and control materials also demonstrate that coating the fabric significantly increases the surface area available for cell attachment and this may also play a role in the advancement of bone formation.

A study of bone ingrowth into Gore-Tex PTFE artificial ligaments suggested that the porosity of the graft fibres, which were 75% air by volume, was key to allowing bone ingrowth. This suggests that the provision of porosity for tissue ingrowth at the bone-graft interface should therefore be considered important when applying bulk materials within the bone tunnel. A stable fixation was found within the bone tunnels up to 18 months after implantation in an ovine model [66]. However, these ligaments were later removed from the market due to problems including loosening and synovitis, along with two documented cases of osteolysis [67]. The implications of these findings as well as those for CaP based materials are considered in the discussion section.

5. Discussion

5.1. Test Methodologies. Differences in methodology render direct quantitative comparisons between these studies complex. Results of biomechanical testing may be influenced by rates of extension, clamping of samples, and chosen angles of flexion. These are not standardised across the testing included in this review. The type of animals used is inconsistent, with rabbit, porcine, and canine models all being common. The number of subjects also varies in animal tests [23, 41].

Time periods for mechanical testing range from two weeks to 52 weeks. The majority of studies last between six and 12 weeks. As the graft healing progresses, differences between treated groups and controls tend to change and do not always follow predictable patterns. Although differences are visible at 6 weeks and sometimes earlier, this may not be a predictor of increased strength later, as shown by the results of Wen et al. [32].

A change in failure mode may be considered a simple means by which to measure at what point the bone tunnel ceases to be the weakest point in the graft. The change in failure mode in biomechanical testing may be therefore considered a useful indicator of improvement over control procedures. It should be noted, however, that if the graft material degrades over time, the mode of failure may change even if the fixation strength has not increased. Examination of failed grafts for assessment of degradation is not commonly carried out.

The extent to which the observed improvements in animal models may relate to changes in outcome for the patient is unclear. Clinical studies regarding this type of application of CaPs are rare. The use of CaPs in resorbable screws, for example, produced improved results in animal models but did not induce a clear improvement in outcome for patients.

5.2. CaPs for Bone Tunnel Healing. Broadly, many of these strategies for the augmentation of bone tunnel healing in soft tissue grafts have been shown to have promising effects. All of the CaP-based materials were judged to have induced improvements in the biological structures forming in the bone tunnels during healing. The improvements included increases in bone mass and changes in the nature of the tissue forming in the tunnels. In the majority of cases, UTS increased (see Table 1), suggesting that the application of these materials increases fixation strength in the short to midterm. However, increases in fixation strength do not always introduce a change in failure mode compared to that observed in the controls. Longer-term studies are desirable in order to properly assess increases in fixation strength in ACL grafting and to provide more detailed information for the planning of clinical trials, through which the benefits of these treatments to the patient may be assessed.

With respect to both increases in fixation strength and changing the mode of failure, the application of CaPs has been shown to produce results comparable to, and in some cases better than, those obtained using materials whose application is more challenging (see Table 2).

When considering which technique to apply for the improvement of intratunnel bone healing, it is important to evaluate the complexity and cost of the method with respect to its efficacy in improving fixation. In the future, the development of devices facilitating the application of GCSF or mesenchymal stem cells, both of which have been shown to produce significant improvements in strength, may be desirable. However, for immediate improvement in bone tunnel healing, the application of existing CaP products such as bone cement seems to significantly improve fixation at a low cost and using a simple procedure and existing approved materials. CaP-based materials were shown to increase fixation strengths and advance healing at the tendon-bone interface. They are widely used biomaterials which are simple to apply and are likely to be among the least expensive of the proposed methods. They have a long-standing safety record and Good Manufacturing Practices (GMP) for them are well established.

The improvements in bone tunnel healing in ACL grafts fixed using CaP is usually attributed to their chemical composition. Bone mineral is a nonstoichiometric form of CaP containing additional elements such as silicon and

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magnesium. Although the exact properties of CaPs depend on the manufacturing processes used to obtain them, they are generally considered to be bioactive. There is insufficient evidence to show whether any particular phase of CaP is preferable in this application.

In addition to the changes in the chemical environment around the healing interface, the application of CaP in injectable or bulk form may offer mechanical advantages in ACL graft fixation. In order for tendon-bone interfacial healing to occur, movement between the two faces must be limited. Where an extensible graft, such as a tendon graft, is inserted into the bone tunnel, the application of bone cement or porous blocks may provide additional fixation proximal to the joint space, limiting intratunnel movement of the graft and facilitating healing. The improvement of bone tunnel healing in this manner may also limit abrasion and wear of the graft due to the restriction of intratunnel graft motion.

While CaP-containing screws are commercially available and clinical evaluation is possible, the application of CaP cement in the bone tunnels does not appear to have been the subject of a clinical study. *In vitro* studies of the application of CaP cements in the bone tunnel have shown significant and consistent improvements in fixation strength and healing of bone tissue in and around the tunnels. This technique could be simply applied and merits further examination.

5.3. Application to Artificial Graft Materials. For the attachment of future artificial grafts, techniques proposed for use in biological grafts may also be of use. Artificial grafts offer the advantage of being able to design both the graft material and the bone-graft interface. Materials which are chosen for their ligament-like properties may be adapted for bone attachment by the application of CaP-based materials. The combination of CaP with PVA, a material which usually resists cell attachment, has been shown to improve its attachment to bone. These techniques for combining polymeric materials with CaP could be adapted to improve the attachment of an artificial ACL graft.

6. Conclusions

This review examines the hypothesis that the application of CaP can improve bone tunnel healing after ACL replacement. In general, ACL-bone tunnel fixation strength can be increased by approximately 100% through the incorporation of CaP and other techniques. The application of growth factors and stem cells merits further investigation but is not immediately clinically applicable. The use of commercially available CaP cements induced changes in all the major indicators: bone formation, biomechanical strength, and mode of failure in biomechanical testing. While the evidence is not conclusive, it suggests that CaP materials perform as well as more complex biologic or cell-based solutions in this application. Studies show that the presence of CaP induces improvements in healing as investigated using histology and medical imaging as well as increases in strength and changes in mode of failure in mechanical testing.

A clinical study into its use to augment fixation and bone tunnel healing in ACL grafts is merited. More specifically, a study linking experimental fixation strengths in a suitable animal model to clinical outcomes in human subjects would be of great benefit, both in establishing the efficacy of this treatment and in helping to establish what parallels, if any, can be drawn between biomechanical testing in animals and clinical results.

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