Potential mechanisms of a periosteum patch as an effective and favourable approach to enhance tendon-bone healing in the human body

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Abstract Tendon-bone healing is a progressive and complex pathophysiological process after tendon graft transplantation into a bone tunnel. A fibrous scar tissue layer forms at the graft-bone interface, which means a weak bonding of the graft in the bone tunnel. Periosteum, a favourable autologous tissue, was confirmed to be effective in promoting tendon-bone healing in the human body. The advantages of a periosteum patch for tendon-bone repair include the fact that this tissue meets the three primary requirements for tissue engineering: a source of progenitor cells, a scaffold for recruiting cells and growth factors, and a source of local growth factors. Furthermore, the periosteum can prevent graft micromotion, alleviate inflammation and deter bone resorption. In this review, we highlight the role of progenitor cells in the periosteum, which contribute to the regeneration of new bone and/or fibrocartilage at the tendon-bone interface. In summary, the periosteum has shown significant potential for use in the enhancement of graft-bone healing. Our investigations may provoke further studies on the management of allograft-bone healing and artificial ligament graft healing using a periosteum patch in future.

Introduction

Tendon healing in a bone tunnel is a priority concern in the orthopaedic and biomedical field when using an autograft or allograft tendon for anterior cruciate ligament (ACL) reconstruction [4, 15]. It is directly related to the long-term

stability of the graft. However, natural tendon-bone healing is poor with a fibrous scar tissue layer formation at the tendon-bone interface [5, 16]. This fibrous layer is extremely weak and will significantly influence the graft stability in the bone tunnel.

Tendon-bone healing is a progressive and complex pathophysiological process after ligament implantation into a bone tunnel. To date, this process of graft-bone healing remains poorly understood. According to a recent report [28], the graft-bone healing process is divided into four stages: (1) inflammation, (2) proliferation, (3) matrix synthesis, and (4) matrix remodelling. In this process, several fundamental factors are possibly involved including: haematoma formation, inflammatory response, fibrocartilage or bone formation at the interface, new vascular ingrowth, matrix degradation by metalloproteinases (MMPs), establishment of collagen fibre continuity and tendon-bone interface motion [28, 41]. Accordingly, many investigations targeting these factors have been applied to promote tendon-bone healing.

Enhancement of tendon-bone healing clinically using periosteum

To date, many approaches have been applied to promote tendon-to-bone healing, including hydroxyapatite [20], bone cement [37], calcium phosphate (CaP) [31], bone morphogenetic protein 2 (BMP-2) [29, 35], BMP-7 [30], platelet-derived growth factor (PDGF) [40], transforming growth factor β_1 (TGF- β_1) [42], granulocyte colony-stimulating factor (GCSF) [36], periosteum [7, 9, 22, 25], bone marrow stromal cells [33] and periosteal progenitor cells [10]. These experimental agents have been demonstrated to be effective in the acceleration of tendon-to-bone

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healing by increasing new bone or fibrocartilage formation and decreasing fibrous scar tissue at the tendon-bone interface. However, only periosteum has been applied clinically to enhance tendon-to-bone healing in the human body. A superior tendon-bone healing process can be achieved when a periosteum patch is sutured onto a tendon graft.

Clinically, Chen et al. [8] applied periosteum into ACL reconstruction for enhancing tendon graft-bone tunnel healing and indicated the periosteum-enveloping hamstring tendon graft achieved a satisfactory effect in ACL reconstruction. In a recent study with a mean follow-up of 4.6 years, Chen et al. [6] investigated 312 patients who received a periosteum-enveloping hamstring tendon graft in ACL reconstruction from 2000 to 2005. The result showed that the median Lysholm knee scores increased from 56 points preoperatively to 95 points after surgery and 93% of patients had a normal or nearly normal rating on the basis of International Knee Documentation Committee (IKDC) assessment. Thus, it can be seen that the envelope of the tendon graft with the periosteum is an effective and favourable way to enhance tendon-bone healing. In this review, the potential mechanisms of a periosteum patch to enhance tendon-bone healing are described from several aspects.

The potential mechanisms of the periosteum in tendon-bone healing

Introduction of progenitor cells

Introduction of progenitor cells to the bone tunnel has been shown to improve the insertion healing of tendon to bone in a rabbit model through formation of fibrocartilaginous attachment at an early stage. Ouyang et al. [33] used a large number of bone marrow stromal cells (BMSCs) in the bone tunnel to enhance tendon-bone healing, and they observed that the group with BMSCs exhibited more perpendicular collagen fibre formation and increased proliferation of cartilage-like cells at the tendon-bone interface at four weeks after surgery. In an ACL reconstruction rabbit model, Lim et al. [26] coated a hamstring tendon graft with mesenchymal stem cells (MSCs). They found large areas of cartilage cells at two weeks and a mature zone of cartilage at eight weeks postoperatively at the tendon-bone interface in the MSC-coated group.

Periosteum, a highly vascularised connective tissue, consists of two layers: an outer layer and an inner layer. The outer layer contains fibroblasts and Sharpey's fibres, and the inner layer contains many osteoprogenic and chondrogenic cells [2, 45]. It has been reported that adult human periosteum-derived cells maintain their chondrogenic potential and can

differentiate into the chondrocytic lineage in vitro [12]. As these chondrocyte precursor cells are located in the inner cambium layer of periosteum, it can be expected that the new cartilage will develop on the side of the cambium layer, rather than on the side of the fibrous layer of the graft [21]. Additionally, it has been found that periosteum can enhance the structural integrity of the tendon-bone interface, when the periosteum is placed between the tendon and bone interface with the cambium layer facing toward the bone [43].

In an investigation comparing the osteogenic capacity between human metaphyseal periosteum-derived cells (hMPCs) and human bone marrow mesenchymal stem cells (hBMSCs), it was found that hMPCs resulted in more robust mineralisation and expressed higher mRNA levels of BMP-2, osteopontin and osteocalcin than hBMSCs in vitro and hMPC showed higher neovascularisation and a higher percentage of mature bone formation than hBMSC in in vivo implantation [11]. To date, periosteum progenitor cells can be isolated and used to enhance graft-bone healing. In a study by Chen et al. [10], histological analysis showed an interface fibrocartilage and new bone formed by photo-encapsulation of BMP-2 and periosteal progenitor cells at six weeks, and biomechanical testing revealed higher maximum pullout strength and stiffness in the experimental group with BMP-2 and periosteal progenitor cells at three and six weeks after tendon transplantation. Collectively, periosteum plays an important role in cell therapy of the tendon-bone healing process, as it affords the osteoprogenic and chondrogenic cells which can form bone or cartilage under appropriate conditions.

Scaffold for cell growth

The recruitment of host cells onto the graft plays an important role in the tendon-bone healing process [23]. As mentioned previously, haematoma forms at the tendon-bone interface, which might influence the recruitment of osteoblasts and/or growth factors from host bone. The periosteum, as a scaffold or matrix, can help recruit host cells and/or growth factors to biologically reinforce the attachment of tendon graft to bone. It has been reported that the periosteum contains a protein called "periostin", which is a disulphide-linked 90 kDa protein secreted by osteoblasts and osteoblast-like cell lines [18]. Horiuchi et al. [18] demonstrated that both purified periostin protein and the periostin-Fc recombinant protein supported attachment and spreading of MC3T3-E1 cells, which suggested that periostin might play a role in the recruitment and attachment of osteoblast precursors in the periosteum. Understanding of these detailed recruitment mechanisms will shed light on the potential use of the corresponding periostin. In future, periostin might be isolated from periosteum and applied in the research of tendon-bone healing.

Addition of growth factors

Periosteum can synthesise many growth factors that regulate cartilage regeneration, including BMP-2, TGF- β_1 and insulin-like growth factor 1 (IGF-1) [32]. These growth factors all together play a crucial role in the new bone formation at the tendon-bone interface. Anderson et al. [3] demonstrated the potential for augmenting tendon healing in an intra-articular bone tunnel using a collagen sponge carrier vehicle containing a mixture of bone-derived proteins, which contained BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, TGF-1, TGF-2, TGF-3 and fibroblast growth factor 1 (FGF-1). BMP-2 plays a key role in the early stage of skeletal development, repair and regeneration by recruiting local sources of progenitors within periosteum and endosteum and by determining their differentiation towards the chondrogenic and osteogenic lineages [44]. Wang et al. [39] demonstrated that deletion of endogenous BMP-2 at the onset of healing abolished periosteum-mediated bone and/or cartilage callus formation. They suggested that the expression of BMP-2 in the periosteum plays a critical role in osteogenic and chondrogenic differentiation of periosteal progenitors during repair. Several investigations [29, 30, 35] have reported on the positive effect of BMP-2 or BMP-7 in the promotion of tendon-bone healing. The BMPs in the periosteum are presumed to play an important role in the enhancement of new bone formation at the tendon-bone interface.

Additionally, TGF- β is a key cytokine in the pathogenesis of tissue fibrosis. As aforementioned, the fibrous scar tissue, which is weaker than the normal fibrocartilaginous insertion, is undesirable at the tendon-bone interface. Interestingly, the addition of TGF- β within the injured site may stimulate extracellular matrix production and trigger the formation of fibrous scar tissue, while blocking the action of TGF- β with gene therapy is a potential approach to avoid scar tissue formation [19]. Diminished macrophage-induced TGF- β production significantly improved morphological and biomechanical properties at the healing tendon-bone interface [17]. However, Yamazaki et al. [42] investigated the effect of TGF- β_1 on intraosseous healing of the flexor tendon autograft in ACL replacement. They found that exogenous administration of TGF- β significantly promoted the bonding strength of the graft to the tunnel wall at three weeks postoperatively, and there were more richly generated collagen fibres in the TGF- β group compared with the control group. Kovacevic et al. [24] studied an osteoconductive CaP matrix with TGF- β_3 at the tendon-bone repair site and found that TGF- β_3 delivery led to greater collagen I expression compared with collagen III at the healing site. They indicated that the administration of TGF- β_3 had a more favourable collagen I to collagen III ration, which might contribute to the establishment of collagen fibre continuity.

Vascularity

Angiogenesis is essential for osteogenesis at the interface between tendon and bone, to facilitate tendon-bone healing [36]. The approaches that improve local vascularity have a potential to augment tendon-bone healing, such as low-intensity pulsed ultrasound and extracorporeal shock wave therapy [27, 38]. Periosteum, a highly vascularised tissue, can contribute to the blood supply of bone and even skeletal muscle [14]. After implantation at the tendon-bone interface, the periosteum is presumed to facilitate new blood vessel ingrowth, which might augment the formation of a fibrocartilage zone between graft and bone tunnel.

Alleviation of bone resorption

Graft micromotion in the bone tunnel may contribute to sustained inflammation caused by repetitive micro-injury at the healing interface. Inflammatory cytokines can promote the expression of MMPs, which accelerate the degradation the bone matrix and influence tendon-bone healing and graft remodelling [1, 13]. Rodeo et al. [34] evaluated the effect of graft-tunnel motion on tendon-bone healing in a rabbit model and observed many osteoclasts at the tunnel apertures. The tunnel apertures had the greatest graft-tunnel motion. These findings suggested that graft-tunnel motion may lead to osteoclast-mediated bone resorption and impair early tendon-bone healing. It is conceivable that a periosteum patch at the graft-bone interface could prevent graft micromotion, alleviate inflammation and avoid bone resorption.

Conclusions and perspectives

In summary, the periosteum has great potential to promote tendon-bone healing by targeting different factors. Here we have summarised several important biological factors which participate in the process of tendon-bone healing. Among these factors, progenitor cells inside the periosteum were reported to play a significant role in the promotion of tendon-bone healing through regenerating bone and/or a fibrocartilage zone. Additionally, growth factors in the periosteum also have a positive effect in the augmentation of tendon-bone healing. Detailed understanding of these growth factors involved in the healing process will shed light on the isolation and use of specific growth factors to enhance tendon-bone healing. Furthermore, the periosteum is presumed to facilitate the regeneration of new blood vessels, which is essential for osteogenesis at the tendon-bone interface. Collectively, it is reasonable to believe that periosteum can induce new bone and fibrocartilage formation at the tendon-bone interface, and our investigations may provoke further studies on the management of

allograft-bone healing and artificial ligament graft healing using a periosteum patch in future.

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