Acta Biomaterialia 42 (2016) 2-17

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

# **Review** article

# Nanoengineered biomaterials for repair and regeneration of orthopedic tissue interfaces

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## ARTICLE INFO

Article history: Received 23 February 2016 Received in revised form 7 June 2016 Accepted 16 June 2016 Available online 17 June 2016

Keywords: Nanomaterials Nanocomposite Tissue engineering Interface tissue Orthopedic tissues Biomaterials

# ABSTRACT

Orthopedic interface tissue engineering aims to mimic the structure and function of soft-to-hard tissue junctions, particularly bone-ligament, bone-tendon, and bone-cartilage interfaces. A range of engineering approaches has been proposed to mimic the gradient architecture, physical properties and chemical characteristics of interface tissues using conventional polymeric biomaterials. Recent developments in nano-materials and nanofabrication technologies introduce a range of synthesis and fabrication tools to effectively engineer the structure and function of native tissue interfaces. In this review, we will focus on nanoengineered strategies used to replicate the structural and functional aspects of native biological tissues for engineering bone-cartilage, bone-ligament, and bone-tendon interfaces. This review will also highlight some of the emerging applications and future potential of nanomaterials and fabrication technologies in engineering tissue interfaces.

#### **Statement of Significance**

A major challenge in engineering interfaces is to control the physical and structural characteristics of an artificial environment. The use of nanomaterials and nanoengineered strategies allow for greater control over the changes in structure and function at molecular and nanometer length scale. This review focuses on advanced nanomaterials and nanofabrication approaches developed to emulate bone-cartilage, bone-ligament, and bone-tendon interface regions. Some of the emerging nanoengineered biomaterials proposed to mimic tissue interfaces are also highlighted.

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The musculoskeletal system, also known as the locomotive system, confers the ability to move through muscular and skeletal attachments. Major components of this system include connective tissues such as bone, tendon, ligament and cartilage. The orthopedic tissue interfaces are classified into i) bone-cartilage ii) bonetendon and iii) bone-ligament, representing a transition from hard to soft tissues (Fig. 1). These interfaces are responsible for the functional interactions between the adjoining tissues and reduce the formation of stress epicenters, which result in the load bearing flexibility. Most musculoskeletal injuries are associated with these interface regions and are common among individuals performing strenuous activities (athletes and military personnel) and as well as the aging population. Typical interventions to heal interfacial tissue injuries involve surgical procedures, suturing the injured tissues and stabilizing via braces, preventing further movement to avoid tearing [1]. However, open surgical interventions suffer major disadvantages such as post-suture scarring, tumor formation, and limited recovery [2]. To overcome these barriers, a range of tissue-engineered approaches are proposed.

A major challenge in engineering interfaces is to control the physical characteristics of an artificial environment in terms of structure and mechanical differences: hard and soft regions. The hard regions usually represent bone tissues, primarily cortical or cancellous bone. Cortical bone is a dense and compact osseous tissue, with a modulus in the range of 16–23 GPa, and forms the outer covering of the bone [3]. Its primary function is to provide stability and protect the internal porous structures. Cancellous bone, however, is relatively soft due to a higher surface area/mass ratio and therefore less dense, with a modulus in the range of 1–2 GPa [4]. Cancellous bone is highly vascularized and metabolically active, and also harbors the bone marrow, which forms the site of

hematopoiesis [5]. The soft regions of the interface are formed from connective tissues such as tendon, ligament, and cartilage. Tendons are fibrous tissues that attach skeletal muscles to bones (Bone-Tendon-Muscle-Tendon-Bone) [6], whereas ligaments link one bone to another and are crucial for joint formation [7]. Both tendon and ligament have a modulus ranging between 0.3 and 0.8 GPa. On the contrary, cartilage is the softest among the three, with a modulus of 0.5–2 MPa, and is primarily responsible for mitigating friction, compressive, and shear forces between bones [8,9].

Engineering tissue interfaces using biomaterials is a challenge due to complex architecture, cell heterogeneity, spatiotemporal distribution of extracellular proteins, and biochemical signals in the native tissue interface [10–12]. For example, tendon is a collagenous tissue connecting bone and muscles. It is made of parallel running collagen fibers and elongated tenocytes, embedded in extrafibrillar matrix [9]. Ligaments are also composed of collagenous fibers loaded with spindle shaped fibroblast cells. The ligament can be distinguished into white and yellow ligament based on the elasticity, the former being inelastic. Apart from connecting bones to bones, ligaments also serve to facilitate the joint movements, protect bone ends, and restrict incompatible movements [13]. For cartilage, the matrix is produced by chondrocytes and is not permeated with blood vessels or nerves, as the nutrient exchange occurs through simple diffusion [14]. Due to an absence of nerves and blood vessels, regeneration of damaged cartilage tissue is severely hampered in ageing and musculoskeletal disorders. In addition, dissimilar properties of bone and other soft tissues make it challenging to mimic the native interface tissue using monolithic biomaterials or conventional fabrication technologies. A range of comprehensive reviews are available that summarize various approaches to engineer interface tissues [15-23].

In this review, we focus on nanoengineered biomaterials and nanofabrication technologies used to mimic interface tissue



Fig. 1. Orthopedic interface tissues include bone-cartilage, tendon-bone, and ligament-bone interfaces. In all these interfaces, a gradual transition in structure, chemical composition, and cell types between the two tissues are observed. The cartilage, tendon, and ligament are collagenous soft tissues, whereas bone consists of mineralized collagen.

structures and properties (Fig. 1). Specifically, we critically evaluated various nanomaterials that have been employed to engineer bone-cartilage, bone-tendon and bone-ligament interfaces. We have also discussed some of the advanced micro- and nanofabrication tools currently used to engineer layered and gradient structures. The scope of this article is to capture the current state of nanomaterial research for orthopedic interface tissue engineering and to identify promising new research directions in the field. Specifically, recent developments that are shaping this emerging field of interface tissue engineering are highlighted, and some of the newly developed nanomaterials that can be used in this area are discussed.

# 2. Nanoengineered biomaterials for orthopedic tissues applications

Nanoengineered biomaterials and nanofabrication technologies have emerged as an alternative to conventional approaches to mimic biological tissues [24-27]. Due to enhanced control over structural, mechanical and chemical properties of nanoengineered materials, cells seeded on or within these 3D scaffold can help in mimicking some of the biological characteristics of native tissue interfaces. For example, various nanofabrication techniques such as electrospinning, and phase separation can provide control over the spatial geometry and biological complexity of the scaffold [28–30]. These nanofabricated scaffolds can control the release of therapeutics to guide cellular behavior [31,32]. Complex geometries such as fibers, spheres, sheets, hollow tubes and nets can be fabricated to mimic some of the biological structures. In this review, we only focus on nanomaterials with one of their dimensions less than 500 nm. Specifically, we will critically evaluate different types of nanomaterials currently used for orthopedic interface regeneration.

A range of ceramic and polymeric nanomaterials has been used for engineering orthopedic tissues including bone, cartilage, tendon, and ligaments [20,27,29]. Ceramic-based nanomaterials including hydroxyapatite, calcium phosphate, nanosilicates, and bioactive glasses have been used for hard tissues such as bone due to their high bioactivity [25–27]. The most commonly explored nanoparticle for bone regeneration is hydroxyapatite (HAp), which has been extensively investigated for orthopedic implants [33–36]. HAp closely resembles biological apatite found in bone tissue, and therefore is a desirable biomaterial for bone regeneration. Other bioactive materials include use of calcium phosphate, bioactive glasses and silicates. Silicate nanoparticles are two-dimensional (2D) nanoparticles that have shown to induce osteogenic differentiation [37,38]. When incorporated into hydrogels, the nanosilicates also increased mechanical properties, which would allow for the material to be applied to bone scaffolds [39-41]. Although not as extensively explored as nHAp, nanosilicates are emerging as a promising material for bone regeneration. These ceramic nanoparticles are composed of complex mineral structures that have shown to bind to surrounding bone and stimulate bone formation. More recently, a range of carbon-based nanomaterials such as carbon nanotubes (CNTs), graphene (G), and nanodiamonds (NDs) have also been explored for bone tissue engineering [42]. Graphene has induced osteogenic differentiation in stem cells [43], and its derivative graphene oxide has also exhibited a similar ability [44].

For soft orthopedic tissues such as cartilage, tendons, and ligaments, only a few types of nanomaterials have been investigated. For cartilage tissue, titanium dioxide ( $TiO_2$ ) nanosheets were explored [45]. These nanosheets were incorporated into an acrylamide hydrogel and the resulting nanocomposite mimicked chemical and physical properties of native articular cartilage. For tendon and ligament tissues, nanofibers are most often used because of the fibrous structure of native tissues. Nanofibers have been fabricated from various polymeric biomaterials including poly (lactic-co-glycolic acid) (PLGA), poly (L-lactic acid) (PLLA), poly (caprolactone) (PCL), and collagen [46–50].

Specifically, for interface tissue engineering, many of the aforementioned nanomaterials have not been investigated and only a few of the conventional nanomaterials are engineered for interface tissue engineering. For example, a range of nanofabrication techniques are used to obtain nanoengineered scaffolds from synthetic and natural polymers including PLGA, PLLA, PCL, collagen, hyaluronic acid, silk, alginate and fibrin. These biomaterials are usually modified for use; in some cases blended with other polymers and nanoparticles (hydroxyapatites, calcium phosphate etc.) to enhance the mechanical properties and bioactive characteristics [25–27]. Specifically, nanoscale topographies obtained by incorporating nanoparticles in the polymeric structure have shown to direct cell fate [51]. In the past couple of decades, the application of nanocomposite materials has progressively surfaced since they can stimulate morphological changes, gene expression, proliferation and differentiation, and mimic the native tissue composition [52].

# 3. Nanoscale technologies to engineer layered and gradient structures

Several fabrication strategies are currently used to engineer orthopedic interface tissues (Fig. 2). The most basic approach involves monolithic scaffolds loaded with growth factors and/or cells [53]. This strategy was commonly used when modeling one tissue type such as bone or cartilage; however when it comes to interface tissues, this strategy cannot represent multiple tissue types. Recently, bi-layered scaffolds have been investigated, where each layer of the scaffold represents a different tissue [54–56]. Although a better representation of the complex interface tissue, this strategy does not account for the interface region [19].

More recently, multi-layered scaffolds consisting of three or more layers have been designed. In this strategy, the middle layer(s) represents the interface region and the outer layers mimic the soft or hard tissue [57-59]. With these layered designs, multiple materials and cell types can be incorporated to mimic the complex architectures of the interface tissues; however, there is not necessarily a smooth transition between the two represented tissues. One of the emerging strategies to mimic interface tissues involves developing a gradient scaffold [19,21,22]. In this approach, a gradual change in the material or the chemical composition is engineered to better recapitulate the native tissue transition. The gradual change can lead to differential expression of cultured cells and give rise to a multifarious environment. Many of the reviewed techniques utilize this gradient approach, and the formation of the chemical or material gradient can be formed through several methods including capillary action, microfluidics, tilt angle, and centrifugation [22,23]. Here, we highlight the gradient and layered nanofabrication techniques as well as nanomaterials that have been employed for orthopedic interface tissues.

#### 4. Nanoengineered bone-cartilage interface

The ultimate aim of interface tissue engineering is to regenerate, augment or repair the damaged interface between the bone and its surrounding tissue. Cartilage injuries are often difficult to treat because damage can occur in both the articular cartilage and the underlying subchondral bone or more specifically the osteochondral interface. Some of the clinically relevant techniques for cartilage regeneration involve osteochondral approaches and include chondrocyte and osteochondral transplantation, as well

# Engineering Approaches to Mimic Structure and Properties of Orthopedic Tissue Interfaces



**Fig. 2.** Engineering approaches for interface tissue engineering. Several strategies including use of monolithic, layered and gradient scaffolds are investigated to mimic the native tissue interfaces. Monolithic scaffolds comprise of one type of biomaterial loaded with cells, whereas layered scaffolds comprise different layers, each representing a single tissue type. Multi-layered scaffolds employ the middle layer, which represents the interface region. The gradient scaffold accounts for the interface region and the smooth transition between two regions.

as debridement of damaged tissues (through arthroscopy). Often, surgical procedures require the removal of the injured bonecartilage region through the creation of an osteochondral defect. Another common surgical procedure for these injures involves microfracture, in which a defect is created by removing calcified cartilage and puncturing the underlying subchondral bone. Small holes are created for bone marrow components including stem cells to fill the defects. Although this procedure often results in less durable and unorganized tissue, it is one of the most common techniques to treat cartilage injuries [60]. Unfortunately, most of these clinical approaches are non-ideal and result in undesired complications to the patient [12,61]. Therefore, recent advancements are focused on minimally invasive approaches to facilitate cartilage regeneration using various polymeric scaffolds such as Hyalograft© (1999) [62], Bioseed© (2001) [63], CaReS<sup>®</sup> (2006) [64], Atelocollagen gel (2007) [65], Cartipatch<sup>®</sup> (2008) [66], Neocart<sup>®</sup> (2009)[67], Chondron<sup>™</sup> (2010) [68], and Novocart<sup>®</sup> (2012) [69] to facilitate cartilage regeneration. Additionally, Tutobone<sup>®</sup>, a bovine-origin bone substitute, and Chondro-Gide® have also claimed to aid in osteogenic repair [70]. However, Tutobone® causes xenogenic reactions, and due to limited clinical data, this product is not a preferred alternative for clinicians [71].

Most of these approaches involve use of a monolithic structure that fails to mimic the anatomical structure or properties. To address this need, various approaches such as multiphasic scaffolds and gradient structures have been investigated to mimic the native architecture [72,73]. For example, bilayered scaffolds have been sought as a key design for regeneration of osteochondral tissues. Some of the commonly employed bilayered structures can be categorized as "independently assembled structures" and "integrated bilayered structures". In independently assembled structures, two discrete scaffolds of bone and cartilage are made individually and then connected before or during implantation [74]. On the contrary, integrated bilayered structures are synthesized as a composite of two different materials [54].

Although the aforementioned strategies are promising, they lack the micro- and nanostructural resemblance to native interface tissues and selection of biomaterials plays an active role in determining the healing outcome [75]. To overcome these problems a range of nanomaterials have been investigated to mimic the structure and mechanical properties of osteochondral interfaces (Table 1). Some of the common nanomaterials that have been exploited for osteochondral interface engineering are nanocomposites composed of PCL, poly (L-glycolic acid) (PGA), or PLGA with hydroxyapatite or calcium phosphate nanoparticles. In addition, some natural materials have also been investigated to mimic the structure of native interface tissue including agarose and collagen

[76,77]. In one study, a binary process of extrusion and electrospinning was used to fabricate a graded, non-woven network of PCL and tricalcium phosphate nanoparticles ( $\beta$ -TCP) [46].  $\beta$ -TCP nanoparticles were injected at varying flow rates, which allowed the formation of a continuous, linear concentration gradient throughout the electrospun PCL matrix. Mouse preosteoblasts were seeded on these scaffolds, and it was observed that the initial rate of cell proliferation decreased in comparison to cells seeded on control tissue culture polystyrene [46]. This decrease was supported by previously documented results suggesting that the decrease in the proliferation was attributed to the onset of differentiation [78]. Four weeks post seeding, a considerable amount of calcium deposit, collagen fiber production, and multilayered cells were observed [46]. Here, the addition of  $\beta$ -TCP nanoparticles aided in directing preosteoblast differentiation.

In addition to  $\beta$ -TCP, hydroxyapatite nanoparticles (nHAp) have also been a popular choice for osteogenic and osteochondral repair strategies. In another study, collagen scaffolds consisting of nHAp crystals were fabricated via a chemical reaction gradient of disodium hydrogen phosphate and calcium chloride [34]. This study, however, did not feature any in vitro validation of cellular response to this graded scaffold. Alginate and agarose gels combined with nHAp were also investigated for osteochondral interface regeneration [79]. The alginate scaffolds did not allow for a uniform distribution of hydroxyapatite; whereas, the agarose gels allowed for uniform distribution of micro- and nano-sized hydroxyapatite (Fig. 3a). Both the micro- and nano-sized hydroxyapatite loaded scaffolds were investigated with interface relevant cells such as deep zone chondrocytes (DZC) and hypertrophic chondrocytes induced by thyroid hormone (DZC + T3). When the agarose/nHAp composite was seeded with the DZC + T3 cells, there was a significant increase in alkaline phosphatase (ALP) activity after 14 days in comparison with the control agarose scaffold. Also on day 14, the addition of nHAp significantly augmented collagen X production and Indian Hedgehog (Ihh) expression (Fig. 3b). The addition of nHAp to the agarose gels, resulted in increased compressive modulus (Fig. 3c). Additionally, there was a positive correlation between collagen content and the compressive modulus in the nHAp scaffold compared to the microHAp and control scaffolds (Fig. 3c). However, no significant effect on the DZC response was observed based on the change in particle size [79]. In the future, both particle sizes could be incorporated into the scaffold since both micro aggregates and nano crystals are found in the native interface tissue [80].

In another study, an unconventional approach was taken by combining nHAp and polyamide 6 (nHAp/PA6) with polyvinyl alcohol/gelatin scaffolds to yield a biphasic scaffold [55]. The polya-

Table 1
Nanoengineered biomaterials for bone-cartilage tissue engineering.

Interface region	Material for bone	Material for cartilage	Significance	Limitations
Bone-Cartilage	Randomly oriented PCL nanofibers and β-TCP nanoparticles	PCL nanofibers	Graded scaffold mimicking structural and compositional properties of natural interface [46]	Only bone specific markers were explored, the cartilage region of the scaffold was not investigated [46]
	Collagen with nHAp	Collagen with nHAp	Compositional and structural gradient created by controlling porosity and calcium phosphate ion concentration [34]	Cellular response to graded scaffold was not explored [34]
	Polyamide 6 and nHAp	Poly vinyl alcohol	Bilayered scaffold supported bone and cartilage regeneration <i>in vitro</i> and <i>in vivo</i> , as well as exhibited sufficient mechanical stability [55]	Each layer was fabricated separately and bone marrow stem cells were differentiated on either region prior to implantation [55]
	PLGA and nHAp	PLGA and nHAp	nHAp enhanced hMSC proliferation and mechanical properties [33]	Scaffolds were investigated individually for bone and cartilage regeneration, not as assembled unit [33]
	Alginate or Agarose with nHAp	Alginate or Agarose	Incorporation of deep zone chondrocytes and nHAp enhanced collagen production and scaffold mechanical strength [79]	Alginate gels did not allow for uniform distribution of nHAp [79] HA particle size did not significantly affect deep zone chondrocyte response [79]
	Agar	PEGDA and nHAp	Injectable and photopolymerizable composite enhancing cartilage anchorage to bone ECM [81]	Limited characterization of the bone region [81]
	Silk Fibroin and nCaP or nHAp	Silk Fibroin	Bilayered scaffolds exhibited increased stability and promoted bone growth and formation of blood vessels [56,86,87] Trilayered scaffolds demonstrated poten- tial for promoting cell differentiation [90]	Long-term <i>in vivo</i> stability remains to be evaluated [56,90]
	Chitosan and nHAp	Chitosan and Silk Fibroin	Four layered, gradient scaffold exhibited range of mechanical properties and initial biocompatibility [91]	Long-term biocompatibility and matrix production remain to be assessed [91]



**Fig. 3.** Nanocomposite scaffolds for osteochondral regeneration. (a) SEM show uniform distribution of nHAp in agarose gel and presence of calcium (Ca) and phosphorous (P) is confirmed by EDS and FTIR analysis. No significant effect of nHAp on elastic modulus and shear modulus is observed. (b) The effect of micro and nano HA particles on GAG and collagen show a significant increase on day 14. Also, the addition of particles leads to a significant increase in ALP activity, production of type X collagen and lhh expression on day 14. (c) The cell-loaded scaffolds have significantly higher mechanical stiffness compared to the acellular scaffolds. Linear correlation analysis shows a positive relationship between GAG content and compressive modulus and shear modulus for all scaffolds. Finally to determine synergistic affects, a significant correlation of GAG + collagen with the nano and micro HA groups is observed. Reproduced with permission [79]. Copyright © 2012, Elsevier B.V.

mide amalgamation aided in an increased stiffness and mimicked mineral structures of native bone tissue, thereby integrating with the osteochondral structure following implantation. A common issue associated with most of the autologous implantation protocols is the chondrocyte extraction from the donor, which can lead to donor-site morbidity and cellular dedifferentiation and accrued damage. The group instead acquired bone marrow stem cells, differentiated them *in vitro* into chondrogenic/osteogenic lineage, and seeded them onto the scaffolds. *In vivo* implantation of these biphasic scaffolds yielded regeneration of the osteochondral region. In addition, the mechanical and structural properties of the scaffold resembled native cartilage and subchondral regions, further warranting its use as an implant material [55].

Recently, an osteochondral scaffold using agar and poly (ethylene glycol) diacrylate (PEGDA) reinforced with nHAp was fabricated [81]. For the bone region, 2% agar loaded with osteoblasts was selected and the cartilaginous phase was fabricated from 15% PEGDA and 0.5% nHAp (pretreated with growth factors) loaded with mesenchymal stem cells. Finally, a thin stainless steel pin was inserted through the center of scaffold in order to assemble the regions as an osteochondral plug. In this study, nHAp was selected to allow for integration between the engineered bone and cartilage regions. Also, nHAp aided in stem cell chondrogenic differentiation within the cartilaginous region. When tested in vivo, the scaffold integrated well with the host bone tissue and demonstrated superior strength, attributed to the addition of hydroxyapatite [81]. In a similar approach, nHAp was incorporated in PLGA scaffolds using thermal phase separation [33]. The introduction of nHAp to the PLGA scaffold increased the compressive modulus from 400 kPa to 600 kPa. The efficacy of the nanocomposites was evaluated in vivo using rat models with osteochondral defects, by delivering mesenchymal stem cells within the scaffold. After four weeks of implantation, the rats showed recovery as highlighted by increased mineralized content, collagen production, and hyaline cartilage formation. The study, however, investigated these scaffolds as individual units for bone-cartilage engineering and not as assembled unit. Further studies on assembled PLGA and PLGA-nHAp should be conducted in order to substantiate these findings [33].

Silk fibroin, a fibrous protein, has been investigated for various tissue engineering approaches due to its high mechanical resilience, tunable degradation characteristics and ability to support cell adhesion and proliferation [82-85]. Specifically, silk based nanomaterials are investigated for bone and cartilage tissue regeneration [56,86–91]. For example, silk-nHAp nanocomposites fabricated using electrospinning were used for controlled release of BMP2 [85]. Silk fibroin have shown to support cell adhesion and proliferation and are investigated for osteochondral tissue engineering [56,86,87]. For example, a bilayered scaffold consisting of porous silk fibroin for the cartilage region and silk-nCaP for the bone region was fabricated using a salt leaching method (Fig. 4a and b) [56]. SEM and micro-CT were performed to characterize the scaffold and confirm the distribution of CaP in the silk matrix (Fig. 4b-d). Although this was a bilayered design, an interface region joined the two distinct layers. The osteochondral regeneration potential of the material was evaluated in a rabbit osteochondral defect model. The subcutaneous implantation of the scaffold resulted in formation of blood vessels and within four-weeks post-implantation, connective tissue was found to adhere to the scaffold surface, which supported the in vivo biocompatibility of the scaffold. Formation of the interfacial region was observed: the silk-nCaP layer induced bone formation and silk promoted type II collagen and glycosaminoglycan production (Fig. 4e). Moreover, the stability of the scaffold addressed the problems of long-term in vivo efficacy [56].

In another study, silk fibroin was incorporated into a trilayered scaffold for osteochondral tissue regeneration [90]. This 3D scaffold

was fabricated via a paraffin microsphere leaching process which allowed for control of the pore size and interconnectivity. The bone and intermediate layer consisted of silk fibroin and nHAp while the top cartilage layer consisted of just silk fibroin that was oriented longitudinally. Adipose-derived stem cells (ADSCs) were seeded onto the bone and cartilage layers of the scaffold and cultured separately in osteoinductive or chondroinductive media. In these *in vitro* microenvironments, ADSCs produced bone and cartilage extracellular matrix proteins in the prospective regions. The intermediate region remained cell-free and prevented the ADSCs within the bone and cartilage regions from mixing with one another. Further studies, specifically *in vivo* models, need to be investigated to observe cellular differentiation capabilities within the scaffold; however, this technique provided a promising trilayered scaffold for osteochondral tissue engineering [90].

Silk fibroin has also been paired with chitosan and nHAp to fabricate multilavered scaffolds for interface tissue engineering [91]. In this study, the top three layers contained a gradient in chitosan and silk fibroin, while the bottom layer contained chitosan and nHAp. In addition, the fabricated layered scaffolds had a gradient in porosity and pore size to mimic native tissue structures. Mechanical properties of the layered scaffolds exhibited an increasing trend in compressive modulus and strength from the first layer containing 25 wt% chitosan and 75 wt% silk fibroin to the bottom layer containing 50 wt% chitosan and 50 wt% nHAp. Chondrocytes were also seeded on the scaffold and proliferate was observed in all four regions of the scaffolds [91]. Although these short-term studies proved initial cell adherence and viability, further studies such as investigating extracellular matrix production are necessary to evaluate scaffold integration and in vivo efficacy.

Although the aforementioned scaffolds have shown potential for bone-cartilage regeneration, most of them involve addition of cells, which could lead to complications in clinical setting. More recently, cell-free scaffolds have gained popularity. These scaffolds stimulate the host environment to differentiate and produce all the necessary components required for regeneration, by the virtue of mechanical and chemical properties of the scaffold [92]. MaioRegen<sup>™</sup> (Fin-Ceramica S.p.A., Faenza, Italy), a cell-free 3D biomimetic graded scaffold for osteochondral tissue engineering, has been investigated in clinical studies [90,92,93]. The tri-layered scaffold fundamentally mimics the cartilage, interface, and subchondral surface of the bone-cartilage region [94]. It is composed of equine-origin type I collagen for the cartilage stimulation, magnesium-enriched nHAp and collagen for the intermediate region, and magnesium supplemented with nHAp for the subchondral bone regeneration. These layers of the scaffold are deantigenated, preventing any immunogenic responses upon engrafting. Furthermore, the scaffold is designed to promote chemotaxis and remodeling and the controlled porosity allows for nutrient exchange [94]. These scaffolds are usually employed for larger osteochondral defects. Also, the simplicity of the onestep surgical procedure involved has been reported to generate favorable outcomes involving minimal follow up and complications [95]. However, a recent study reported inconsequential osteochondral recovery using this biomimetic scaffold [92]. Additionally, a major ambiguity in these studies is the absence of gold standards, therefore, no comparisons are made through controls. More recently, another approach for cartilage regeneration was explored using a decellularized cartilage based scaffold [96]. Preliminary results with bone marrow derived mesenchymal stem cells indicated increased expression of osteogenic and chondrogenic markers without any external growth factors. In the future, this scaffold could be used in an osteochondral defect in vivo and in the complex environment regional differentiation may be possible [96]. Although the presented nanomaterial approaches for



**Fig. 4.** Bilayered nanocomposite scaffolds for osteochondral regeneration. (a) Synthesis of bilayered silk/silk-nCaP scaffold (top) and subsequent implantation in rabbit model (bottom). (b) SEM and EDS analysis of the layered scaffold investigates presence of calcium in different zones (Z1-4). (c) CaP distribution investigated using micro-CT, showing two distinct layers – silk layer (brown) and composite layer (blue). (d) Quantitative analysis of porosity distribution shows homogeneous and interconnected porous network in each layer. (e) *In vivo* studies reveal type II collagen (red) production in the silk layer of the scaffold (bottom right) but is not present in the control defect (top right); the S refers the scaffold and the arrow points to newly formed tissue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Reproduced with permission [56] *Copyright* © 2015, *Elsevier B.V.* 

treating bone-cartilage injuries are promising, further studies must be done to evaluate these scaffolds as true candidates to replace the clinical standard treatments. In addition, many of the approaches only incorporate nanomaterials into the bone region of the scaffold to improve the mechanical properties of the material while only a few explored the nanomaterials' bioactivity. Future studies could study the effect of incorporating nanomaterials into both regions of the scaffold for both structural stability and bioactivity.

# 5. Nanoengineered bone-tendon interface

Tendons attach muscles to bones and structurally they are very similar to ligaments. Tendon damage or tears occur most often in joints such as the shoulder or knee and the injury can be severe enough to damage the bone-tendon interface. Traditionally, these injuries are stabilized through surgery, which as previously discussed can lead to more complications. Most of the well-known strategies for tendon repair rely on artificial tendon grafts and implantation of allogenic or xenogeneic grafts such as GraftJacket<sup>®</sup> [97], TissueMend<sup>®</sup> [98], Restore<sup>™</sup> [99], Permacol<sup>™</sup> [100], and CuffPatch<sup>™</sup> [101]. Given the source and xenogeneicity of these transplants, patients can suffer from severe immunogenic conse-

quences. Moreover, it has been shown that the tendons regenerated through these scaffolds have inferior elastic moduli in comparison to the native tissue [102]. The native bone-tendon region is composed of mineralized fibrocartilage on the bone side and nonmineralized fibrocartilage on the tendon side. In the native tissue, this region exists as a gradient structure and recent approaches (Table 2) focus on mimicking these graded structures using different cells or nanomaterials [16].

Interfacial regions of the bone-tendon are commonly organized through longitudinally collocated collagen fibers, which consist of cells organized throughout the mesh [9]. In one study, this concept was explored to design a PLGA nanofibrous scaffold and revealed that the fiber alignment affects the cell morphology; fibroblasts cultured on aligned fibers were better spread whereas the random fibers had atypical polygonal morphology [48]. This was supported by  $\alpha 2$ ,  $\beta 1$  and  $\alpha V$  integrin expression of the cultured rotator cuff fibroblasts. Also, it was noted that these symmetrically organized fibers had higher elastic modulus (0.34 GPa) in comparison to the randomly aligned fibers (0.107 GPa). In vitro, the randomly aligned fibers exhibited an accelerated degradation profile. This study also substantiates that the cells in these soft tissue regions are able to recognize the fiber alignment and direct their proliferation. Nanofibers are also more physiologically relevant than microfibers and have better biomimetic potential [48].

Table 2				
Nanocomposite	biomaterials	for	bone-tendon	interfaces.

Interface region	Material for bone	Material for tendon	Significance	Limitations
Bone-Tendon	Random PLGA nanofibers	Aligned PLGA nanofibers	Orientation of nanofibers directed fibroblast morphology. Mechanical properties similar to native tissue were achieved [48] Gradient composition and fiber ori- entation are important for mimicking native tissue[50]	Mechanical properties after long- term <i>in vitro</i> studies were not evaluated[48] Scaffolds were not evaluated <i>in vivo</i> [48,50]
	Plasma-treated PLGA and gelatin-coated PCL nanofibers with calcium phosphate coating	Plasma-treated PLGA and gelatin-coated PCL nanofibers	Calcium phosphate coating created a gradient enhancing cell proliferation [104]	Cell differentiability was not evaluated on the graded scaffold [104]
	PLGA nanofibers with HA nanocomposite	PLGA nanofibers	Fiber structure and mineralization are necessary to mimic native tissue [47]	Scaffolds were not investigated in vitro or in vivo [47]
	Calcified Fibrocartilage and subchondral bone region: collagen with HA nanocomposite	Tendon layer: collagen Uncalcified fibrocartilage region: collagen and chondroitin sulfate nanocomposite	Multi-layer human collagen based scaffold was fabricated which mimicked native tissue physical and mechanical properties [57]	The scaffold's ability to support cell differentiation was not assessed; pre- differentiated cells were seeded on each layer of the scaffold [57]

PLGA nanofibers have also been engineered with mineral gradients of hydroxyapatite to create a controlled environment for the osteogenic differentiation of cells. One study highlighted the use of fibrous scaffolds in conjunction with a mineralization pattern to promote osteogenesis [103]. To further this, rotator cuff fibroblasts could be co-cultured to create a bone-tendon transitional environment, which could potentially regenerate the interfacial region. In a similar study, a mineralized graded scaffold formed on plasma treated PLGA and gelatin coated PCL electrospun fibers was investigated (Fig. 5a and b) [104]. The calcium phosphate coating was chosen to enhance cell proliferation and differentiation. MC3T3 cells seeded onto these scaffolds showed a linear correlation with increased calcium phosphate (Fig. 5c). In addition, the gradation in mineral content along the nanofibers affected the mechanical properties. The mineral gradient led to a spatial gradient of the scaffold's stiffness; increasing the levels of mineral on the gradient led to an increase in modulus which also suggested a stiffening effect on the nanofibers (Fig. 5d) [104]. A recent study, however, indicated that calcium phosphates may inhibit osteogenic differentiation due to their low crystallinity and high rate of dissolution [103].

The native tissue architecture of the bone-tendon interface exhibits a change in fiber alignment; highly organized collagen fibers in a tendon, whereas the transition represents more inconsistency in alignment [105]. This information prompted a study where cell alignment on systematically organized fibers was observed to be similar to an aligned soft tissue (such as tendon) and that transition to a disoriented mesh would prove beneficial in bone-tendon interface regeneration [50]. The mechanical behavior of this graded electrospun scaffold was also found to be very similar to the bone-tendon structure. Similar studies have been conducted using nanohydroxyapatite (nHAp) [47,106]. A combinatorial approach of controlling mineral density, chemical factors, and fiber organization could prove influential in the search of an improved design. Yet another innovative study employed natural collagen fibers complexed with nHAp to echo the natural bonetendon interface [57]. A multilayered scaffold was created using: (i) collagen crosslinked network (tendon region), (ii) collagen and chondroitin sulfate matrix (uncalcified fibrocartilage region), (iii) low nHAp concentration in collagen matrix (calcified fibrocartilage region) and (iv) high nHAp concentration in collagen (bone region) (Fig. 6a) [57]. The addition of nHAp created distinct morphologies in the bone and calcified fibrocartilage regions and the pore size of the bone region decreased due to the HA crystals penetrating the collagen matrix (Fig. 6b). Along with the pore size, the mechanical properties of each layer varied and mimicked that of natural tissue (Fig. 6b). From the tendon layer to the bone layer, an increase in elastic modulus was observed. Finally, human cells were cultured on the layered scaffold; human fibroblasts, chondrocytes, and osteoblasts were seeded on the tendon, fibrocartilage, and bone regions respectively. On each layer, a uniform distribution of cells was observed as investigated by fluorescence imaging and SEM (Fig. 6c) [57]. The study accords a novel design strategy that could be established as an important prototype to engineer native interface tissues.

Overall, there has been a remarkable advancement in the design and material organization strategies; however, a major overhaul of our understanding of the native interfacial architecture is required in order to proceed to major breakthroughs. Also, locally existing mineral gradients in native tissues vary in the magnitude of micrometers [107], whereas existing standards do not offer this feature.

# 6. Nanoengineered bone-ligament interface

Ligaments form the nexus between two bones. The functional anatomy of the ligament still remains to be fully understood, hence, one of the most challenging interfacial regions to regenerate. Anterior cruciate ligament (ACL) injuries are the most common cause of ligament impairment. Topical approaches for treatment involve autograft and allograft implantation, which as previously mentioned, undertake severe complications [108]. Alternatively, the use of artificial ligaments has been proclaimed to treat these injuries; however, a mechanical mismatch between implant and native tissue has been sourced to result in arthritis [109,110]. More recently, tissue engineering strategies involving the use of various synthetic and natural polymers in conjunction with cells and growth factors have shown promise in repair of bone-ligament injuries (Table 3).

Nanofibrous structures and gradients are the most investigated scaffolds for bone-ligament repair, which commonly involve the use of biological nanofillers such as nanohydroxyapatite (nHAp). A co-electrospun scaffold to mimic the mechanical and biological composition of the bone-ligament interface was fabricated in one study (Fig. 7a) [111]. Specifically, a continuous graded scaffold containing nHAp-poly(caprolactone) (nHAp-PCL) for bone regeneration and poly(ester urethane)-urea elastomer (PUEER/PUR) to restore ligament was fabricated. The scaffold was subjected to simulated body fluid (SBF) to facilitate the mineral deposition (Fig. 7b). SEM images revealed nHAp presence on PCL fibers and the smooth morphology of the PUR fibers. In addition, when subjected to SBF,



**Fig. 5.** Graded nanofibers for bone-tendon repair. (a) Graded calcium phosphate (CaP) coating added to the gelatin-coated PCL and plasma treated PLGA nanofibers. (b) SEM images of CaP graded PLGA nanofibers at different distances along the scaffold. (c) Fluorescent images of MC3T3-E1 cells seeded on different regions of CaP graded gelatin-coated PCL nanofibers. The higher concentration of CaP, result in enhanced cell attachment and spreading. (d) The unmineralized portion of the scaffold exhibits the highest strain and lower modulus compared to mineralized region. Reproduced with permission [104] *Copyright* © 2009 American Chemical Society.

the gradient in mineral content was apparent (Fig. 7c). The moduli observed for the gradient was two-to-three orders of magnitude lower than that of human ligament tissue (Fig. 7e); however the moduli of PUR and PCL separately are comparable; therefore their mechanical properties should sustain regeneration. They further investigated maturation and differentiation of bone marrow stem cells (BMSCs) on these scaffolds (Fig. 7d) [111,112]. The mineral gradient significantly suppressed ALP mRNA production, while enhancing BMP-2 and OPN expression, thereby, stimulating osteoblastic differentiation in BMSCs (Fig. 7f) [112]. Although promising results are obtained from this study, the response of cells and formation of ECM need to be investigated under dynamic conditions to mimic *in vivo* conditions.

In another study, mesenchymal stem cells (MSCs) were cultured on poly (lactide-co-glycolic acid) (PLGA) nanofibers [113]. The cellloaded nanofibers were subjected to bFGF treatment and then mechanically stimulated in a bioreactor. The study showed a synergistic effect of mechanical stimulation upon co-stimulation along with the chemical treatment, evidenced by an increase in type I and III collagen, tenascin, and fibronectin expression. Although this brief study encourages the use of these nanofibers for ACL treatment, it fails to highlight the bone and transition regions of the scaffold design [113].

A two-spinneret system was developed to fabricate a nanofiber scaffold gradient in another study [114–116]. The nanofibers were fabricated from PCL modified with amorphous calcium phosphate nanoparticles (nACP). PCL scaffolds have shown to support osteogenesis and addition of calcium phosphate can further promote deposition of mineralized ECM. When tested with mouse preosteoblasts (MC3T3-E1), regions of the scaffold containing higher amounts of nACP exhibited significant cell adhesion and proliferation compared to PCL. This trend is similar to the noncalcified to calcified transition in the native ligament-bone interfaces [116]. This study, however, did not consider any structural aspects of native tissue and additional experiments to investigate the effect of mechanical loading on ECM production need to be investigated.

In another study, nanofibrous scaffolds with a structural and mineral gradient were fabricated using PLGA and nHAp [106]. The mineral gradient was established by varying the nHAp concentration while structural organization was controlled using fiber alignment, random against aligned fibers. Alternative to nanofiber scaffolds, a woven, nHAp hybrid silk scaffold, seeded with osteo-



**Fig. 6.** Multilayered scaffolds for bone-tendon repair. (a) Four layered scaffold (left) and material specification with corresponding represented tissue (right). (b) SEM images of different layers of the scaffold indicate a porous and interconnected network. A gradual change in pore size and mechanical stiffness was observed from the tendon layer to the bone layer. (c) Live/dead staining after 7 days indicates a uniform distribution of cells in different layers. Fibroblasts were seeded on the tendon layer, chondrocytes on the two middle regions, and osteoblasts on the bone layer. SEM images of cells in different layers after 14 days (bottom) indicate formation of ECM. Reproduced with permission [57] *Copyright* © 2014 John Wiley & Sons, Inc.

blasts, BMSCs, and fibroblasts to represent the bone, interface, and ligament regions, respectively was fabricated [117]. Superior mechanical properties and biocompatible characteristics have made silk a promising material for interface regeneration strategies [118,119]. The presence of osteoblasts and fibroblasts in the adjacent regions, allowed BMSCs to differentiate into both the lineages, thereby giving rise to a transitional region. This hybrid silk scaffold supported a tri-lineage environment and the researchers aimed to construct grafts for ACL treatments [117].

Another nontraditional approach to bone-ligament regeneration involved fabrication of two- and three-dimensional electrospun scaffolds [120]. PCL and PLGA were respectively chosen for the aligned and random portions of the scaffold and the transition region contained both materials (Fig. 8a). BMSCs were cultured on both regions of the 2D mesh and results showed cells mimicked the environment of the regions; cells were aligned on the PCL region and were randomly oriented on the PLGA region (Fig. 8b). Previously, fabrication of a 3D cylindrical scaffold had not been extensively studied; however, here it proved to be a potential solution for bone-ligament regeneration. The presented methods offer promising nanoengineered strategies for bone-ligament regeneration, and developing nano- and microfabrication techniques will allow for greater control of fabrication processes for mimicking interface tissues.

Table	3
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Nanoscale biomaterials to engineer bone-ligament interface.

Interface region	Material for bone	Material for ligament	Significance	Limitations
Bone-Ligament	nHAp-PCL nanofibers	PEUUR nanofibers	Co-electrospun continuously graded mesh exhibited mechanical and physical properties similar to native tissue. hMSC differentiation [111,112]	Cellular response to the scaffold and the formation of ECM were not investigated under dynamic conditions to mimic <i>in vivo</i> conditions [111,112]
	PLGA nanofibers with bFGF	Aligned PLGA nanofibers	Chemical and mechanical stimuli enhanced hMSC proliferation and differentiation respectively [113]	Bone and transition regions of the scaffold were not well-characterized [113]
	PCL nanofibers with nACP	PCL nanofibers	Two-spinneret approach for direct nanofiber gradient without additional modification [116]	Scaffold ECM production and mechanical loading were not evaluated [116]
	PLGA randomly oriented nanofibers	PCL aligned nanofibers	Electrospun 2D meshes and 3D cylindrical composites with controlled fiber orientation, diameter, chemistry, and mechanical properties [120]	The 3D cylindrical composite lacked mechanical strength in the aligned PCL region [120]



**Fig. 7.** Graded electrospun scaffold for bone-ligament regeneration. (a) The smooth transition present between the natural bone and ligament. (b) Graded scaffold consists of nHAp-PCL fibers (green), PUR fibers (red) and an interface region (middle). (c) SEM images before and after treatment with SBF. (d) F-actin stained BMSCs cultured on unmineralized (top) and mineralized (bottom) scaffolds after 28 days. (e) The tensile modulus for the mineralized nHAp-PCL fibers is significantly higher than all other fibers (<sup>\*</sup>p = 0.002). (f) BMP-2 expression and ALP activity is not significantly different between unmineralized and mineralized fibers. Reproduced with permission [111,112] *Copyright* © 2011, 2012 Elsevier B.V.

# 7. Emerging trends and techniques

A recent surge in the development of new bioactive nanomaterials and our understanding of the complex relationships between nanomaterial structure and properties have resulted in the expansion of smart and functional biomaterials [29]. The use of nanomaterials for biomedical applications is rapidly expanding and promising new improvements in the area of tissue engineering have been demonstrated [25–27]. For example, a range of new bioactive nanomaterials such as 2D nanomaterials, metal oxides, and ceramic nanoparticles have been developed to control and trigger stem cell differentiation into different lineages (Fig. 9a). Some of the new categories of nanomaterials that have shown promise in the area of orthopedic tissue engineering include use of graphene oxides [43,44], synthetic silicates [37,41], and titanium dioxide ( $TiO_2$ ) [45]. Due to the exponential growth in nanomaterial development in recent years, it is expected to provide a wider selection of nanomaterials with custom physical, chemical, and biological characteristics that can be tailored for various biomedical and biotechnological applications. Most of these new nanoma-



Fig. 8. Electrospun mesh for bone-ligament repair. (a) The electrospun scaffold consists of three regions, random PLGA fibers, aligned PCL fibers and random PCL fibers. SEM images reveal microstructure of each region. (b) BMSCs cultured on electrospun scaffolds readily adhere and spread on the fibrous scaffold. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Reproduced with permission [120] Copyright © 2014 John Wiley & Sons, Inc.

terials have not been investigated yet for interface tissue engineering and there is tremendous potential to design and develop smart nanomaterials for engineering orthopedic tissue interfaces.

A potential avenue for evaluating various nanomaterials for interface tissue engineering is use of high-throughput screening (Fig. 9b). 3D biomaterial microarrays hold enormous promise for regenerative medicine because of their ability to quickly optimize the right combination of biomaterials, cells, and the ECM environment for certain applications [121,122]. The use of 3D microarrays can, if optimized correctly, result in more than 1000-fold reduction in biomaterials and cells consumption when engineering optimal nanomaterials combinations, which makes these miniaturized systems very attractive for interface tissue engineering.

In addition, recent efforts on designing functional biomaterials also focus on developing multicomponent system consisting of two or more nanomaterials [26,27]. These multicomponent systems have the ability to exhibit distinct characteristics. For example, magnesium oxide (MgO) nanoparticles coupled with nHAp and PLLA, have shown to increase osteoblast adhesion and proliferation and also provide antimicrobial properties [123,124]. Although these multicomponent nanomaterials have shown increased osteoblast proliferation and promise for bone tissue applications, future studies need to be conducted in order for these materials to be applied to interface tissue engineering. Additionally, most of these new developed strategies are evaluated for bone-related applications and very limited studies focus on evaluating these new nanomaterials for other orthopedic tissues including cartilage, tendon, and ligament [125–127]. Thus there is a need to investigate these next generations of biomaterials for interface tissue engineering.

Another emerging approach in tissue engineering is additive manufacturing [128–132]. Conventional techniques used to fabricate scaffolds for interface tissues include salt leaching, electrospinning, phase separation (thermally induced), freeze drying, gas foaming, emulsification, and solvent casting and particulate leaching (SCPL). Many of these techniques use salts, porogens, and organic solvents, which result in limited cellular infiltration and encapsulation. To overcome these limitations, recent approaches have shifted towards additive manufacturing. Some of the additive manufacturing approaches that can be used to engineer interface tissues include 3D printing [133–135], stereolithography, air pressure aided deposition [136,137], and robotic dispensing [138–140]. These free-form prototyping techniques face problems of bio-printability, which limit the use of printing cells with the scaffolds.

Recently developed bioprinting techniques can be used to engineer orthopedic tissue interfaces (Fig. 9c). So far, bioprinting has only been used to print one or two types of tissue; however with the emergence of new and improved bioinks, there is a possibility



**Fig. 9.** Emerging trends in interface tissue engineering. (a) Bioactive nanomaterials such as ceramic, metal oxides and 2D nanomaterials have potential to control and trigger cellular processes. (b) Microarray printing technology can be used to screen nanomaterials library in a high-throughput manner. (c) Bioprinting techniques can be used to engineer layered scaffolds for orthopedic tissue interfaces. (d) The use of a 3D bioprinter can mimic native tissue architecture with high spatiotemporal control of cells and physical/chemical clues.

to print layered and/or gradient tissues [141]. 3D microarray systems can be used to generate layered/gradient-like tissue interfaces and such multilayered microgel arrays can be used for high-throughput screening [121]. We believe that the development of new high-throughput technologies for studying stem cell behavior within multilayered materials would significantly advance the field of interface tissue engineering.

Recently, 3D bioprinting can be used to print three different cell types using laver-by-laver deposition of custom bioinks (Fig. 9d). For example, an alginate-collagen bioink revealed that cells could be localized in predetermined positions without compromising cell viability [142]. Although the cells were not printed with a bioink, this study proves the viability of printing cells to control cell placement in a 3D tissue construct. Another aspect of 3D printing that makes it appealing for engineering interface tissues is that its resolution would allow for gradients to be fabricated not only in the xand y-directions, but also in the z-direction [143,144]. In addition, a dual nozzle syringe on the printer would make it possible to print multiple biomaterials at the same time. Previously, gradients have been fabricated using a gradient maker and mixing chamber in which the volume of different materials are controlled and added at different rates to create zones [145]. A 3D bioprinter can mimic native tissue architecture with high spatiotemporal control. Overall, 3D bioprinting will provide an improved strategy for engineering interface tissues and advance the field of tissue engineering.

# 8. Conclusion

Interface tissue engineering has seen remarkable progress in the past decade with continued improvements from autologous transplantation to rapid prototyping of different biomaterials. Nanomaterials such as nanofibrous and nanocomposite scaffolds loaded with hydroxyapatite, calcium phosphate, or aragonite are attractive scaffolding materials, since they can control and direct cell fate and tune the formation of ECM. Additionally, nanomaterials can be customized to control the degradation profile to facilitate tissue regeneration. These nanoengineered scaffolds and nanofabrication techniques have the potential to minimize surgical interventions and overcome the complexities associated with donor site morbidity. Additionally, nanomaterials can be tuned to contain binding sites, growth factors, and signaling proteins, which are important for chemical transductions. As new bioactive materials and fabrication technologies are developing, it is possible to mimic some of the physical and chemical properties of native tissues interfaces. Specifically, the emergence of bioactive nanomaterials offers promise for directing cell behavior. Although, these nanofabricated constructs mimic the interfacial regions efficiently. their clinical translation has not been achieved due to lack of strong clinical data. In addition, in order to create less invasive surgical procedures to treat injuries at interface tissues, these nanomaterial strategies need a minimally invasive delivery method such as an injection. Some nanomaterials strategies have emerged that allow for injection and can provide a facile and simple approach for clinical applications [41,146]. However, the effect of shear stress on cell viability and cellular processes need detailed investigation using small and large animal models. Another challenge with nanomaterials is assessing their short-term and longterm toxicity, especially with the newly developed nanomaterials. Long-term accumulation of nanomaterials in body as well as inflammatory reaction due to degradation products of nanomaterials needs more critical evaluation. Overall, nanoengineered scaffolds have become important components in interface tissue engineering since they offer an improvement in terms of design and control at the molecular level, although further studies must be conducted to evaluate their clinical relevance. The fieldwork has led to exciting advancements, and there is potential for nanomaterial-based scaffolds to emerge as new treatment methods for orthopedic interface tissue injuries.

# Acknowledgements

LC would like to acknowledge financial support from Texas A&M University Diversity Fellowship. AKG would like to acknowledge funding support from Texas Engineering Experiment Station and Texas A&M University Seed Grant.

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