

Commentary

Current state of cartilage tissue engineering

Richard Tuli, Wan-Ju Li and Rocky S Tuan

Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Department of Health and Human Services, National Institutes of Health, Bethesda, Maryland, USA

Correspondence: Rocky S Tuan (e-mail: Tuanr@mail.nih.gov)

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Abstract

Damage to cartilage is of great clinical consequence given the tissue's limited intrinsic potential for healing. Current treatments for cartilage repair are less than satisfactory, and rarely restore full function or return the tissue to its native normal state. The rapidly emerging field of tissue engineering holds great promise for the generation of functional cartilage tissue substitutes. The general approach involves a biocompatible, structurally and mechanically sound scaffold, with an appropriate cell source, which is loaded with bioactive molecules that promote cellular differentiation and/or maturation. This review highlights aspects of current progress in cartilage tissue engineering.

Keywords: biomaterials, cartilage, mesenchymal progenitor cells, tissue engineering

Introduction

Cartilage degeneration caused by congenital abnormalities or disease and trauma is of great clinical consequence, given the limited intrinsic healing potential of the tissue. Because of the lack of blood supply and subsequent wound-healing response, damage to cartilage alone, or chondral lesions, results in an incomplete attempt at repair by local chondrocytes. Full-thickness articular cartilage damage, or osteochondral lesions, allow for the normal inflammatory response, but result in inferior fibrocartilage formation. To prevent progressive joint degeneration in diseases such as osteoarthritis, surgical intervention is often the only option. In spite of the success of total joint replacement, treatments for repair of cartilage damage are often less than satisfactory, and rarely restore full function or return the tissue to its native normal state.

The rapidly emerging field of tissue engineering holds great promise for the generation of functional tissue substitutes, including cartilage, by engineering tissue constructs *in vitro* for subsequent implantation *in vivo*. The basic principle is to utilize a biocompatible, structurally and mechanically sound scaffold that is seeded with an

appropriate cell source, and is loaded with bioactive molecules to promote cellular differentiation and/or maturation. Although recent progress has been made in engineering cartilage of various shapes and sizes for cosmetic purposes [1], the challenges of engineering a weight-bearing tissue, such as articular cartilage that consists of multiphasic cellular architecture, are significant. There have been a number of successful approaches to tissue engineer cartilage, including the use of natural and synthetic biomaterial scaffolds, allogeneic and autologous sources of mature chondrocytes and chondroprogenitor cells, chondroinductive growth factors, such as the transforming growth factor- β s (TGF- β s), and combinations thereof. We have highlighted here some of the current advances in cartilage tissue engineering.

Cell-scaffold composites

Given the lack of cell retention when cell suspensions are directly transplanted at the cartilage defect site [2], as well as potential donor site morbidity associated with procedures that utilize a periosteal flap to increase cellular retention of such cell suspensions [3], porous three-dimensional scaffolds are increasingly being used to facilitate cellular attachment while providing superior

mechanical properties. Although recent studies utilizing hyaluronan- and collagen-based natural biopolymeric scaffolds have shown promise, lot inconsistency, combined with the potential for immunogenic problems, has prompted investigators to focus mainly on synthetic polymer-based scaffolds, such as the poly- α -hydroxy esters. Freed *et al.* have shown that the rates of chondrocyte proliferation and deposition of cartilage-specific glycosaminoglycans are significantly higher on polyglycolic acid (PGA)-based scaffolds as compared to poly(L)lactic acid (PLA)-based scaffolds [4], while both polymers have been shown to promote proteoglycan synthesis at higher rates than collagen scaffolds [5]. The ability to promote chondrocyte proliferation, maturation, and differentiation, and the superior mechanical properties of polyester-based biodegradable polymers strongly suggests the feasibility of their application in cartilage repair. Interestingly, the copolymer, polyD,L-lactide-co-glycolide (PLGA), was recently shown to be most effective in promoting osteoblastic cell attachment with increased $\alpha 2$, $\alpha 5$, and $\beta 1$ integrin expression [6], suggesting that patterned scaffolds consisting of different synthetic polymers may be considered for biphasic tissue engineering, such as an osteochondral construct.

Injectable materials are also being considered for cartilage tissue engineering applications to circumvent the need for invasive surgery, as would be required with prefabricated scaffolds. The naturally derived polysaccharide gel, alginate, has been successfully shown to support cell retention and the chondrocytic phenotype by maintaining cell shape through encapsulation [7]; however, its inferior biomechanical properties as well as concerns over its immunogenicity have raised biocompatibility issues [8]. In a promising study, a chondrocyte-fibrin suspension injected into critical-sized cartilage defects *in vivo*, resulted in the successful deposition of cartilage-specific extracellular matrix molecules and improved healing as compared to untreated control defects [9]. Additionally, using an injectable, biocompatible, and biodegradable polyethylene oxide-based gel for the encapsulation of isolated chondrocytes, Sims *et al.* [10] observed that, when injected subcutaneously into nude mice, the gel scaffold maintained three-dimensional spatial support, promoted chondrocyte proliferation, and facilitated production of a well-formed cartilaginous matrix [10]. However, the excellent biocompatibility, resorbability, and malleability of polyethylene oxide-based hydrogels, give way to their inferior biomechanical properties; consequently, optimal applicability of such materials is likely to be limited to cosmetic surgical procedures, such as craniofacial surgeries. A novel approach to significantly improve mechanical strength involves amalgamation of a biodegradable polymer with alginate as a scaffold to support chondrocyte or mesenchymal stem cell (MSC) differentiation and transplantation – the polymer providing adequate support to

the mechanically unstable gel, thereby facilitating *in vivo* implantation. For example, Caterson *et al.* have utilized a three-dimensional biodegradable PLA-alginate amalgam scaffold in combination with TGF- $\beta 1$ to support the attachment/retention and chondrogenic differentiation of MSCs, while conferring mechanical stability to the construct [11]. Marijnissen *et al.* compared demineralized bone matrix to a PLA-PGA fleece, both used in conjunction with alginate gel, in their capacity to support the chondrocytic phenotype *in vivo*. Structural homogeneity as well as the number of collagen type II positive cells was found to be higher in the PLA-PGA-alginate constructs [12], once again confirming the well-suited applicability of such biodegradable polymers to the repair of cartilage defects.

Another biomimetic approach is to develop nanoscopic biodegradable scaffolds as cell delivery vehicles that have structural and morphological properties similar to those of native extracellular matrix, thereby mimicking the cells' natural environment while providing structural stability [13]. Li *et al.* have demonstrated the ability of electrospun poly- ϵ -caprolactone-based nanofibrous scaffolds to support the chondrocytic phenotype of fetal bovine chondrocytes [14] and the chondrogenic induction and maintenance of TGF- $\beta 1$ treated MSCs (unpublished data). Remarkably, this poly- ϵ -caprolactone-based nanofibrous scaffold also appears to support the adipogenic and osteogenic induction of human MSCs (unpublished data), suggesting its potential application for multiphasic tissue engineering, such as craniofacial remodeling and other therapeutic procedures of skeletal regeneration.

To be considered for tissue engineering applications, the architecture of the scaffold should ideally mimic that of the native tissue to be repaired; additionally, this implantable scaffold should be suited to facilitate infiltration, attachment, proliferation, and differentiation of the desired, individual cell type. Recent efforts have been devoted to designing non-uniform, heterogeneous scaffolds for clinical applications that require multiphasic tissue engineering, such as for the repair of osteochondral lesions. For example, utilizing bovine articular chondrocytes seeded onto a PGA mesh scaffold and sutured to a PLGA-polyethylene glycol foam loaded with bovine periosteal cells, Schaefer *et al.* observed well-developed cartilaginous and bone-like tissues, which maintained their individual phenotypes during the composite culture and formed a well-defined cartilage-bone interface [15]. Taking a different design approach to fabricate a construct which mimics the relevant features of the tissue to be repaired, Sherwood *et al.* have used the TheriForm™ three-dimensional printing process to develop a unique, heterogeneous scaffold with variable material composition, porosity, and mechanical properties to suit its design for the repair of osteochondral lesions [16], while also allowing for versatility in overall shape. Chondrocytes preferentially attached

to the “cartilage-like” portion of the scaffold and formed cartilage *in vitro*, while the cloverleaf “bone-like” portion maintained a tensile strength comparable to that of native trabecular bone. Interestingly, for procedures such as repair of osteochondral lesions, such a complex construct would have the advantage of promoting ingrowth of native bone tissue, while optimizing the transition zone to prevent delamination of tissues at the cartilage-bone interface. Clinical feasibility awaits *in vivo* studies to assess repair of osteochondral lesions.

The promise of mesenchymal progenitor or stem cells

Although the use of chondrocytes for applications of cartilage tissue engineering is prevalent, concerns associated with donor site morbidity, cell dedifferentiation, and the limited life span of these cells have brought the usage of MPCs or MSCs to the forefront of such applications [17]. MPCs can be found resident within a host of musculoskeletal and connective tissues, and the multipotential nature of MPCs makes them theoretically ideal candidates for repair of cartilage defects, especially those that also involve subchondral bone. Gao *et al.* [18] tested this hypothesis by attempting repair of osteochondral defects using a two-phase composite material to mimic natural tissue geometry that is composed of injectable calcium phosphate (ICP) and a hyaluronan derivative loaded with MPCs. At 12 weeks postimplantation, the grafted composite displayed distinct zones of repair tissue, including columnar arrays of chondrocyte-like cells, which integrated with surrounding native cartilage and the new bone tissue that formed within the ICP. Interestingly, however, Solchaga *et al.* [19] reported that a fibronectin-coated, hyaluronan-based sponge was able to organize and facilitate the reparative response following implantation within an osteochondral defect, even without preloading the scaffold with autologous bone marrow as a source of MPCs [19], suggesting an enhancement of the natural repair response by scaffold alone. The combination of scaffold preloaded with bone marrow was not found to significantly benefit the long-term repair process, but did, however, allow for a more homogeneous filling of the scaffold, ultimately promoting integration of the newly formed cartilage repair tissue with the host tissue.

Recent efforts have also been directed towards the *in vitro* prefabrication of MPC-based cartilage and osteochondral constructs prior to implantation. Using a novel one-step procedure, Noth *et al.* [20] have successfully developed an *in vitro* engineered cartilage construct by press-coating MPCs onto a PLA scaffold. Following a 3-week period of culture in chondrogenic conditions, the construct displayed a hyaline cartilage-like morphology, with organized and spatially distinct zones positive for collagen type II and link protein. Using human trabecular bone-derived MPCs [21, 22] and a PLA scaffold, our labo-

ratory has recently constructed a single-unit osteochondral plug consisting of a collagen type II-positive, but collagen type I-negative, hyaline cartilage-like layer adherent to, and overlying, a dense, mineralized bone-like component, and separated by a well-demarcated interface similar to that of native tissue (submitted for publication). During the course of long-term co-culture, the chondrogenic and osteogenic cells continued to differentiate and maintain their specific phenotypes. The use of only two starting materials, autologous MPCs and a PLA scaffold, provide the added benefits of minimizing handling, while maximizing biocompatibility for repair of osteochondral defects.

Conclusion and future direction

While it is recognized that functional, biologically engineered tissue substitutes represent a highly promising alternative solution to current methods of cartilage repair, key challenges remain to be addressed. For example, implantation of a cell-scaffold into a hostile, tissue-degradative environment, such as for treatment of a focal osteoarthritis lesion, would seem imprudent given the potentially rapid breakdown of matrix components that would ensue. A potentially attractive solution would be a combined gene therapy and tissue engineering approach. For example, Kafienah *et al.* [23] implanted cells transduced with tissue inhibitor of metalloproteinases-1 to protect the cells from the degradative effects of matrix metalloproteinases induced by cytokines, such as IL-1 and tumor necrosis factor- α . Future research should thus be aimed at investigating and evaluating tissue-engineering approaches to cartilage repair in disease-compromised animal models to gain a better understanding of clinically feasible designs. The results of such studies should have direct therapeutic applications, and should also provide a model system for the study of normal and pathological cartilage tissues.

Competing interests

None declared.

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Correspondence

Rocky S. Tuan, Ph.D., Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Department of Health and Human Services, National Institutes of Health, Building 50, Room 1503, 50 South Drive, MSC 8022, Bethesda, MD 20892-8022, USA. Tel: +1 301 451 6854, Fax: +1 301 402 2724, e-mail: Tuanr@mail.nih.gov