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REVIEW ARTICLE

Clinical translation of stem cells: insight for cartilage therapies

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Abstract

The limited regenerative capacity of articular cartilage and deficiencies of current treatments have motivated the investigation of new repair technologies. *In vitro* cartilage generation using primary cell sources is limited by cell availability and expansion potential. Pluripotent stem cells possess the capacity for chondrocytic differentiation and extended expansion, providing a potential future solution to cell-based cartilage regeneration. However, despite successes in producing cartilage using adult and embryonic stem cells, the translation of these technologies to the clinic has been severely limited. This review discusses recent advances in stem cell-based cartilage tissue engineering and the major current limitations to clinical translation of these products. Concerns regarding appropriate animal models and studies, stem cell manufacturing, and relevant regulatory processes and guidelines will be addressed. Understanding the significant hurdles limiting the clinical use of stem cell-based cartilage may guide future developments in the fields of tissue engineering and regenerative medicine.

Keywords

Cartilage tissue engineering, clinical trials, osteoarthritis, regenerative medicine, stem cell treatments

History

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Introduction

Articular cartilage has a unique extracellular matrix (ECM) composition and structure enabling it to withstand the high loads of joints such as the knee. Trauma or degenerative conditions, such as osteoarthritis, can permanently damage cartilage, which has an intrinsically limited healing capacity, and subsequently reduce joint mobility. The tremendous burden resulting from arthritis, projected to affect 67 million individuals by 2030 (Hootman & Helmick, 2006), has spurred the investigation of new treatment strategies. Disadvantages of current surgical treatments for cartilage healing – including donor site morbidity and biomechanically inferior fibrocartilage formation (Horas et al., 2003) – have prompted the investigation of tissue engineering, which aims to produce neocartilage that can function in the native environment and thus restore joint function. The resulting new treatments should avoid requiring the patient's own cells or tissue, discourage multiple surgeries, and emphasize functionality upon implantation by recapitulating articular cartilage properties.

Cartilage regenerative medicine encompasses approaches to repair, regenerate or treat defects or pathologies via stem cell use or induction. Examples include stem cell injections or chemo-attraction of neighboring stem cells. The classical tissue engineering approach involves scaffolds, cells, and signals. Tissue engineering and regenerative medicine overlap where tissue implants are engineered using stem cells. Cell sourcing issues, including limited expansion potential (Darling & Athanasiou, 2005) and scarcity of primary chondrocytes, hinder clinical translation of cartilage tissue engineering technologies, which often require large cell numbers. This limitation spurs the investigation of stem cell-based cartilage tissue engineering. Chemical (e.g. TGF-β superfamily growth factors) and mechanical (e.g. compressive or tensile loading) signals are typically used to differentiate stem cells down a chondrocytic lineage, before and/or after scaffold placement.

Human embryonic stem cells (hESCs) constitute a promising cell source that can provide large cell numbers and circumvent primary cell sourcing issues. hESCs and induced pluripotent stem cells (iPSCs) are immortal and pluripotent, but require extensive manipulation prior to obtaining chondrogenic cells. Multipotent adult mesenchymal stem cells (MSCs) are also investigated for cartilage regeneration. MSCs, with the capacity to differentiate into cartilage, have been isolated from bone marrow, adipose tissue (ASCs), umbilical cord matrix, skin and synovial tissue. In an allogeneic clinical treatment, employing MSCs or hESCs would eliminate the need for harvesting patient cells and reduce the treatment turnaround time. In addition, production of allogeneic stem cell banks increases the product's commercial potential.

To determine the success of stem cell-based neocartilage engineering, the native tissue must provide benchmarks. Hyaline articular cartilage consists of a solid phase, interstitial fluid and mobile ions. The solid phase mainly consists of ECM, including collagen (50–75%/dw) and proteoglycans

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(15–30%/dw); chondrocytes occupy 1–5% of the tissue by volume (Hu & Athanasiou, 2003; Little et al., 2011). The rest is primarily water and dissolved solutes, comprising 60–85% of cartilage by wet weight (Mow et al., 1992). Glycosaminoglycans (GAGs) associate with aggrecan molecules and confer a large negative charge to cartilage, sequestering water and creating osmotic pressure that resists compressive loads, giving cartilage a compressive modulus up to 2×10 MPa (Schinagl et al., 1997). Because the fluid phase bears the initial load, hydrostatic pressure is generated during joint loading. As water leaves the joint, the compressive load is transferred to the solid phase. Cartilage exhibits a strong structure–function relationship: the mechanical properties are intimately linked to ECM composition and organization. Therefore, reproducing the matrix is crucial for attaining native cartilage properties.

Although there have been successes in stem cell-based cartilage tissue engineering, clinical translation of these technologies for cartilage repair and regeneration remains severely limited. According to ClinicalTrials.gov, a global registry of public and private clinical trials, 21 clinical trials intended for stem cell-based cartilage repair are currently registered as of 6/10/2013 (search terms: ''stem cells'' and ''cartilage''). All of these trials are MSC-based, with 24% of these trials using allogeneic MSCs. Aside from ethical and political concerns, there are major hurdles to translating stem cell products: sufficient pre-clinical data availability; production and facility expenses; and government product regulations. This review will discuss the successes and shortcomings of the current field of stem cell-based neocartilage engineering, clinical translation requirements for these technologies and how these requirements can inform the field's future directions. It will also address acceptable cellular processing methods, product implantation and manufacturing obstacles of stem cell-based cartilage products.

Stem cell-based cartilage regeneration

Tissue engineering and regenerative medicine

Various stem cell-based tissue engineering methods have independently achieved promising biochemical and mechanical properties toward those of native cartilage (Huang et al., 2010; Li et al., 2010; Meyer et al., 2011). However, the lack of an optimal stimulation regimen limits the progression of neotissues to the clinic. Tables 1 and 2 provide an overview of the most commonly used biochemical and mechanical stimulation methods in tissue engineering of neocartilage from stem cell sources (see ''Search strategy and selection criteria''). Cartilage tissue engineers must reach a consensus regarding the best magnitude, application time, duration and frequency of these signals. Rather than investigating singular chemical or mechanical conditions, a systematic evaluation of these factors would define a successful platform upon which to build, allowing the field to ultimately achieve neotissues matching native articular cartilage. Researchers investigating multiple stimuli must consider that increasing the number of manipulations needed to differentiate stem cells before implantation increases translational barriers (e.g. scale-up).

Scaffold and scaffold-free approaches

A wide variety of scaffolds have been employed to assist chondrogenic differentiation of stem cells, including agarose, collagen and hyaluronic acid (Chung & Burdick, 2008; Leddy et al., 2004) (Tables 1 and 2). MSCs seeded in agarose produce neocartilages with compressive properties nearing 45% of native tissue (Athanasiou et al., 1991; Huang et al., 2009). Native cartilage ECM scaffolds (i.e. collagen type II) can produce better cartilage than synthetic scaffolds (Bosnakovski et al., 2006). These results illustrate the importance of recapitulating an appropriate stem cell niche that promotes and maintains a chondrocytic phenotype. Certain scaffolds that promote cell attachment and spreading may result in altered cellular phenotype (Li et al., 2003). Cartilage's avascularity may also prevent removal of toxic scaffold by-products, ultimately hampering clinical translation.

Circumventing scaffold-associated issues, scaffold-free approaches may recapitulate cartilage morphogenesis (Ofek et al., 2008), generating neotissues with compressive properties nearly 65% of native tissue values (Ando et al., 2007). Scaffold-free technologies avoid scaffold-associated stress-shielding and reduced cell–cell communication. Scaffold-free methods help maintain a chondrocytic phenotype, which is particularly important concerning stem cell plasticity. Without scaffolds that hinder cell proliferation or matrix deposition, scaffold-free neocartilage may integrate better post-implantation. However, scaffold-free approaches require a comparatively high number of cells, which can be addressed by refining chondrodifferentiation strategies to produce more viable cells.

Stem cell sourcing for tissue engineering

Endogenous therapies are based on the recruitment of the body's own stem cells to the cartilage defect, whereas exogenous approaches use stem cells that are first prepared *in vitro* and then delivered *in situ*. Endogenous stem cells have been shown to migrate to defect sites (or home) and are efficacious in initiating cartilage healing *in vivo* (Erggelet et al., 2007; Lee et al., 2010). Exogenous stem cell injection can similarly initiate repair; however, it is unclear whether injected or recruited cells are the major contributors to tissue repair. More information regarding the role of endogenous stem cells can be found elsewhere (Gerter et al., 2012).

MSC chondrodifferentiation can be achieved using scaffolds and growth factors to up-regulate aggrecan and collagen II gene expression, indicating their potential for neocartilage formation toward cartilage tissue engineering (Diekman et al., 2009) (Table 1). Due to their immunoprivilege, MSC use in cartilage repair may alleviate concerns of a host immune response (Beyth et al., 2005). On the other hand, autologous MSC-based cartilage therapies must take into account that MSCs exhibit age-dependent limitations, with MSC numbers declining with age (Caplan, 2007). Applying chondrodifferentiation protocols to autologous MSCs that decrease in availability has broad implications for an aging population prone to cartilage afflictions; thus, allogeneic sources may be best in these cases.

Table 1. Mesenchymal stem cell (MSC) chondrodifferentiation techniques.

Source	Stimulus	Level	Culture method	Reference
Growth factor stimulation				
BM	TGF-β3	20 ng/mL	Collagen-GAG	(Matsiko et al., 2012)
	TGF-β3	10 ng/mL	PCL	(Abrahamsson et al., 2010)
	TGF-β3	10 ng/mL	Hyaluronic acid	(Erickson et al., 2009)
	TGF-β3; BMP-2	10 ng/mL; 50 ng/mL	Pellet	(Perrier et al., 2011)
	TGF-β3; TGF-β1	10 ng/mL; 20 ng/mL	Gelatin/albumin	(Mohan et al., 2009)
	TGF-β1	10 ng/mL	Agarose, alginate, gelatin	(Awad et al., 2004)
	TGF-β1	10 ng/mL	PEG	(Nguyen et al., 2011)
	TGF-β1	10 ng/mL	Gelatin	(Solorio et al., 2012)
	TGF-β1	10 ng/mL	Collagen-GAG	(Liang et al., 2010)
	TGF-β1	10 ng/mL	Hyaluronic acid	(Toh et al., 2012)
	TGF-β1; IGF-1	10 ng/mL; 100 ng/mL	PLLA	(Janjanin et al., 2008)
Syn	TGF-β3; BMP-2	10 ng/mL; 500 ng/mL	Chitosan/alginate	(Qi et al., 2011)
	TGF-β1; TGF-β3; BMP-2	10 ng/mL; 10 ng/mL; 10 ng/mL	Gellan gum	(Fan et al., 2010)
	BMP-2	100 ng/mL	Pellet	(Ando et al., 2007)
AD	TGF-β1; IGF-1	10 ng/mL; 100 ng/mL	PLGA/chitosan	(Zhang et al., 2013)
	TGF-β1; BMP-6	10 ng/mL; 100 ng/mL	Monolayer, alginate	(Lee et al., 2013)
	TGF-β1; BMP-6	10 ng/mL; 10 ng/mL	Pellet	(He & Pei, 2013)
	TGF-β1; BMP-2	10 ng/mL; 50 ng/mL	Pellet, hyaluronic acid	(Yoon et al., 2011)
	TGF-β3; BMP-6	10 ng/mL; 10 ng/mL	Pellet	(Hildner et al., 2010)
AD, BM	TGF-β2; BMP-7	5 ng/mL; 100 ng/mL	Pellet	(Kim & Im, 2009)
Mechanical stimulation				
BM	Compression	4 h/day, 3 days @ 10% strain, 0.1, 0.5, or 1 Hz	Fibrin	(Pelaez et al., 2009)
	Compression; shear	1 h/day, 5 days/week, 3 weeks @ 10–20%, 1 Hz; ±25°	Fibrin-polyurethane	(Schatti et al., 2011)
	Ultrasound	10 m/12 h, 1 or 4 weeks @ 200 mW/cm^2, 1 MHz	Fibrin-hyaluronic acid	(Choi et al., 2013)
BM, AD	HP	4 h/day, 21 days @ 7.5 MPa, 1 Hz	Agarose	(Puetzer et al., 2012)
AD	Compression	4 h/day, 7 days @ 5%, 1 Hz	Chitosan/gelatin	(Li et al., 2012)
Syn	HP	1 hr @ 1 or 5 MPa, 0.5 Hz	Alginate	(Sakao et al., 2008)
Combined growth factor and mechanical stimulation				
BM	TGF-β1 Compression	0, 1, 10 ng/mL 1 h/day, 7 days @ 10–20%, 1 Hz	Fibrin-polyurethane	(Li et al., 2010)
	TGF-β1 Compression	10 ng/mL 4 h/day, 7 days @ 8 MPa, 0.33 Hz	Hyaluronan/gelatin	(Angele et al., 2004)
	TGF-β1 Compression	10 ng/mL 4 h/day, 3, 4, or 7 days @ 10%, 1 Hz	Pellet, agarose	(Huang et al., 2004)
	TGF-β3 Compression	10 ng/mL 1 h/day, 3 or 6 weeks @ 10%, 1 Hz	Agarose	(Thorpe et al., 2010)
	TGF-β3 Compression	10 ng/mL 1 or 4 h/day, 5 days/week, 3 weeks @ 10%, 1 Hz	Agarose	(Huang et al., 2010)
	TGF-β3 Compression	10 ng/mL 180 m/day, 5 days @ 10%, 1 Hz	Agarose	(Mauck et al., 2007)
	TGF-β3 Compression	10 ng/mL 1.5 h/6 h, 24 h/day, 8 days @ 15%, 1 Hz	Alginate	(Campbell et al., 2006)
	TGF-β1 Tension	10 ng/mL 7 days @ 10%, 1 Hz	Collagen-GAG	(McMahon et al., 2008)
	TGF-β3 HP	10 ng/mL 4 h/day, 5 day/week, 3 weeks @ 10 MPa, 1 Hz	Agarose	(Steward et al., 2013)
	TGF-β3 HP	10 ng/mL 4 h/day, 3, 7, or 14 days @ 0.1, 1, 10 MPa, 1 Hz	Pellet	(Miyanishi et al., 2006)
	TGF-β3 Fluid shear	1 ng/mL 4 weeks @ 100μL/m/fibre mesh	Chitosan/PBTA	(Alves da Silva et al., 2011)
	TGF-β1 Ultrasound	10 ng/mL 20 or 40 m/day, 7 days @ 30mW/cm^2, 1.5 M Hz	Pellet or Hyaluronan/Gelatin	(Schumann et al., 2006)
	TGF-β1 Ultrasound	10 ng/mL 20 m/day, 7, 14, 21, 28 days @ 200mW/cm^2, 1 M Hz	Monolayer	(Lai et al., 2010)
	TGF-β1 Ultrasound	10 ng/mL 20 m/day, 1 or 2 weeks @ 200mW/cm^2, 1 M Hz	Alginate	(Lee et al., 2007)
AD	TGF-β1 Compression	5 ng/mL 30 m/2 h, 16 h/day, 14 days @ 15%, 0.3 Hz	PEGDA	(Steinmetz & Bryant, 2011)
	TGF-β1 HP	10 ng/mL 1 week @ 0.5 MPa, 0.5 Hz	Collagen	(Ogawa, 2009)
	TGF-β1 HP	10 ng/mL 4 h/day, 7 days @ 5 MPa, 0.5 Hz	Pellet	(Safshekan et al., 2012)
	TGF-β3 HP	10 ng/mL 4 h/day, 5 day/week, 3 weeks @ 0.4, 5 MPa, 0.5 Hz	Gellan gum	(Correia et al., 2012)
AD, Syn	TGF-β3 HP	1,10 ng/mL 4 h/day, 14 days @ 10 MPa, 1 Hz	Pellet	(Vinardell et al., 2012)

Commonly used growth factors and mechanical stimuli for inducing MSC chondrodifferentiation in cartilage tissue engineering. Most growth factors are applied continuously throughout culture. References selected based on PubMed search, as described in ''Search strategy and selection criteria''. BM: bone marrow; Syn: synovium-derived; TGF: transforming growth factor; BMP: bone morphogenetic protein; IGF: insulin-like growth factor; HP: hydrostatic pressure; GAG: glycosaminoglycan; PCL: poly-ε-caprolactone; PEG: poly(ethylene glycol); PLLA: poly(L-lactic acid); PLGA: poly(L-glutamic acid); PBTA: poly(butylene terephthalate adipate); PEGDA: poly(ethylene glycol) diacrylate.

Compared to MSC chondrodifferentiation studies, there is a dearth of studies regarding hESC differentiation to chondroprogenitors (Table 2). No direct, systematic comparison between the growth factor-induced chondrocytic potential of these sources has been performed, and a study that determines the differentiation efficiency of MSCs versus hESCs given similar stimuli would greatly direct the field. A single study illustrates hESC-derived MSCs as more sensitive to mechanical loading than MSCs (Terraciano et al., 2007). Knowledge obtained from hESC research could be applied

Table 2. Pluripotent stem cell chondrodifferentiation techniques.

Source	Stimulus	Level	Culture method	Reference
Growth factor stimulation				
ESCs	TGF-β1	10 ng/mL	Monolayer	(Hwang et al., 2008a)
	TGF-β1	10 ng/mL	Micromass	(Toh et al., 2010)
	TGF-β1; BMP-2	10 ng/mL; 10 ng/mL	Gelatin	(Alfred et al., 2011)
	TGF-β1; BMP-2	10 ng/mL; 25 ng/mL	Pellet	(Hwang et al., 2006)
	TGF-β1; TGF-β3; IGF-1; BMP-2	10 ng/mL; 10 ng/mL; 100 ng/mL; 10 ng/mL	EB	(Koay et al., 2007)
	TGF-β1; BMP-2	2 ng/mL; 2, 10 ng/mL	EB	(zur Nieden et al., 2005)
	TGF-β1; BMP-7	10 ng/mL; 300 ng/mL	Pellet	(Nakagawa et al., 2009)
	TGF-β3	10 ng/mL	EB	(Jukes et al., 2009)
	TGF-β3; BMP-2	10 ng/mL; 10 ng/mL	EB	(Bai et al., 2010)
	BMP-4	0.5 ng/mL; 30–100 ng/mL	EB; monolayer	(Craft et al., 2013)
ESCs, iPSCs	TGF-β1; BMP-2	10 ng/mL; 10 ng/mL	Micromass	(Yamashita et al., 2013)
iPSCs	BMP-4; TGF-β3	50 ng/mL; 10 ng/mL	Micromass, pellet	(Diekman et al., 2012)
	BMP-2	100 ng/mL	Micromass	(Guzzo et al., 2013)
	BMP-2	100 ng/mL	EB, agarose, PCL	(Kim et al., 2011)
Growth factor and mechanical stimulation				
MSCs, ESCs	TGF-β1	10 ng/mL	Pellet, PEGDA	(Terraciano et al., 2007)
	Compression	1, 2, 2.5, 4 h/day, 7, 14, 21 days @ 10%, 1 Hz		

Commonly used stimuli for inducing pluripotent stem cell chondrodifferentiation in cartilage tissue engineering. Most growth factors are applied continuously throughout culture. References selected based on PubMed search, as described in ''Search strategy and selection criteria''. ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; TGF: transforming growth factor; BMP: bone morphogenetic protein; IGF: insulin-like growth factor; EB: embryoid body; PCL: polycaprolactone; PEGDA: poly(ethylene glycol)diacrylate.

toward the use of iPSCs for cartilage tissue engineering, shifting the entire field into the realm of personalized medicine (Diekman et al., 2012).

Chemical and mechanical stimulation

TGF-β, BMP-6 and dexamethasone, among other soluble factors, have been widely used to chondrodifferentiate MSCs and hESCs (Estes et al., 2006; Hwang et al., 2008b; Koay et al., 2007; Mehlhorn et al., 2007) (Tables 1 and 2). While these potent stimuli enhance neocartilage properties, their dosing and temporal use requires optimization. Applying an abundance of chemical stimuli should be avoided, as excess use can result in unwanted differentiation, overgrowth of tissue or undesirable hypertrophy of cells. Furthermore, in implanted constructs, residual growth factors may adversely impact the native joint environment. Alternatively, the stem cells within the implant may not survive in the joint without *in vitro* growth factor levels.

Mechanical stimuli – such as dynamic compression, hydrostatic pressure and tension – have been applied as effective chondrodifferentiation agents (Baker et al., 2011; Kisiday et al., 2009) (Tables 1 and 2). Applied at physiologic levels, these stimuli mimic natural joint biomechanics. For example, dynamic compression mimics the cyclic loading of the joint and elicits cellular biosynthesis. As with chemical stimulation, mechanics-based protocols differ in loading magnitude, duration, time of application, duty cycle and frequency. Variations in loading protocols and equipment prevent the direct comparison of successful studies, thus limiting optimization and ultimately hampering the progression of the field toward clinical applications. As with chemical stimuli, commercialization of neotissues generated using mechanical loading are susceptible to scale-up considerations, requiring large bioreactor development.

Despite successes in using chemical and mechanical stimuli independently, the interactive effects and overall benefit of combined treatments are difficult to decipher. The importance of mechanical stimuli in chondrodifferentiation is itself a contentious topic. It is postulated that mechanical loads are transduced through mechanotransductive elements (e.g. ion channels, integrin receptors) to affect chondrogenesis; an alternative hypothesis is that loading may simply allow for improved fluid-borne transport of chondro-inductive chemicals. Thus, mechanical and chemical stimulation regimens are difficult to decouple. However, understanding the difference has broader implications for translation, as the use of soluble factors is more amenable to scale-up considerations than mechanical regimens. The discovery and use of chemical equivalents to mechanical stimuli may alleviate these concerns and facilitate translation of stem cell-based neocartilage.

Evaluation of stem cell-based cartilage therapies in animal models and veterinary medicine

Existing animal models

The USA Food and Drug Administration (FDA) guidance document for products intended to repair or replace knee cartilage acknowledges that ''no perfect animal model of articular cartilage injury exists'' (FDA, 2011a). Both small and large animal models should be used to show safety, efficacy and durability of response. Small animal models are less expensive and can provide an initial indication of safety (e.g. biocompatibility) and efficacy of stem cell-based treatments; the rabbit is the most popular model for cartilage defects. However, it is generally accepted that spontaneous healing of cartilage defects occurs in rabbits, potentially confounding the results of such studies, but not in large animals or humans. Therefore, a large animal model (e.g. sheep, horse) is necessary to further evaluate efficacy,

especially if the product is intended for load-bearing joints such as the knee (Guo et al., 2004; McIlwraith et al., 2011; Zscharnack et al., 2010).

Lasting repair is shown by durability studies, best conducted in large animals that better approximate the biomechanics and scale of human diarthrodial joints. Large animals also offer the potential for long-term follow-up. Furthermore, efficacy in small animals does not necessarily translate to large animals. For example, in 12/12 rabbits with full-thickness cartilage defects, an ASC/fibrin scaffold treatment resulted in hyaline-like repair (Dragoo et al., 2007). In horses, a similar defect model and treatment yielded positive results initially, but durability was not seen 8 months after implantation (Wilke et al., 2007). These discrepancies demonstrate that preclinical animal studies may require multiple animal models to evaluate durable cartilage repair.

Results from animal studies are most meaningful when a model is chosen to reflect human pathology or injury and when a standard set of data are reported. While many defect and pathology models have been generated in large animals, their fidelity to human conditions varies and few, if any, are widely adopted. This is especially true for diseases like osteoarthritis where complex tissue interactions exist. To facilitate comparison of such models, a minimum, standardized set of data should be reported; while the FDA's guidance document suggests a list of parameters, not all published studies report them. For example, the FDA guidance document suggests reporting the animal model (i.e. joint size and load, age, skeletal maturity) and articular cartilage defect type (i.e. location, size, depth), as well as a description of methods regarding defect preparation, gross and histological assessments, and mechanical evaluations. Better characterization of appropriate animal models is needed, and publications reporting a standard set of parameters are expected to improve model development.

Stem cells also present challenges for choosing an animal model. For a proposed therapy containing human cells, animal studies should either: (1) use the intended human cells in combination with immunosuppressives or (2) use animal-derived cells that are analogous to ''the ultimate clinical product in phenotype and biologic activity'' (FDA, 2011a). Immunosuppressives are costly, may increase morbidity and potentially influence treatment efficacy. The potency of analogous, animal-derived cells may also differ from the final human product. When an intended human product contains autologous cells, should autologous cells also be necessary in an animal study, given that there is no evidence that autologous cells perform differently than allogeneic cells in animal models? Considering that allogeneic cells would reduce cost and number of procedures, are allogeneic cells sufficient to prove efficacy, even if the intended product is autologous? Ultimately, some proposed treatments may require evaluation via multiple large animal models prior to first-in-human studies.

Clinical use of stem cells to treat cartilage disease in veterinary medicine

The promise of stem cell therapy has driven an industry in veterinary use. Pre-clinical studies in dogs and horses have facilitated rapid translation of stem cell therapies into veterinary medicine, creating a gap between human and veterinary markets. ASCs have been commercially available for use in veterinary clinics since 2003; commercial and academic institutions offer fat and bone marrow processing for autologous re-implantation to treat osteoarthritis, bone fractures and tendon or ligament injuries. Stem cell therapies have also been developed for other diseases, including renal failure in cats. While one company reports more than 8000 animals treated with their stem cell product, there is notably little published information documenting efficacy in clinical veterinary patients.

Blinded, controlled trials of a commercially available product administered by intra-articular injection have demonstrated improvement in clinical signs (e.g. lameness, pain and range of motion) associated with elbow and coxofemoral osteoarthritis in dogs (Black et al., 2007, 2008). There is no evidence that the treatment repaired or regenerated cartilage. Intra-articular injection of the same product did not result in significant improvement in clinical, histological or biochemical parameters associated with cartilage repair in an induced model of osteoarthritis in horses (Frisbie et al., 2009). Such discrepancies illustrate the need for more rigorous examination of stem cell therapies in veterinary medicine to yield greater insight on the role of stem cells in modulating idiopathic osteoarthritis.

For translation to humans, companies commercializing veterinary products must be incentivized to publish their methods and results. Such transparency may minimize the gap between human and veterinary markets, ultimately accelerating the establishment of stem cell-based cartilage repair for human afflictions. The existing use of stem cells in veterinary medicine creates an exciting opportunity for collaboration between veterinarians and physicians to advance the treatment of injury and disease in both human and animal patients alike.

Limitations of bench-to-bedside translation of cartilage therapies

Culture and processing methods

Commercialization of current chondrodifferentiation techniques may be subject to scale-up limitations, excessive costs and regulatory hurdles (as discussed in the ''Regulation of Stem Cell-Based Cartilage Products'' section). For instance, hydrostatic pressure bioreactors are easily scaled-up, unlike direct compression bioreactors which are more challenging. Commercialization of stem cell therapies is subject to the design of novel bioreactors that can sustain large-scale production of stem cells while using minimal space and maintaining low costs (Alfred et al., 2011; Marolt et al., 2006; Tao et al., 2011). The challenge is that development of culturing techniques potentially distinct from those used in basic research might be required to maintain the desired cellular phenotype to keep the product efficacious on an industrial scale. For example, clinical-grade flow sorting may be used to ensure homogeneity of the product when used in a Good Manufacturing Practices (GMP)-rated facility that follows appropriate manufacturing guidelines (Hare et al., 2009; Jung et al., 2012; Koç et al., 2000). Large-scale use of exogenous growth factors may prove prohibitively expensive.

In addition to cost and production logistics, regulations also delay translation. Examples of regulatory issues include techniques that employ bacterial plasmids or viral vector-mediated genetic engineering. While chondrogenic genes may be up-regulated effectively using such methods, extensive regulatory oversight due to biosafety concerns exist (e.g. a 15-year follow-up of all treated patients by the FDA and recombinant advisory committee, RAC). Thus, due to unique circumstances related to stem cells, feasibility at the industrial level should be considered even during the research phase for tissue-engineered cartilage products.

Product implantation

According to the FDA, implantation in humans must be preceded by sufficient evidence for differentiation efficiency, integration, safety, and long-term viability and functionality. Appropriate differentiation and efficacy must be demonstrated both *in vitro* and *in vivo* as the initial ''proof-of-concept'' stage (Figure 1). Then, strong data supporting biosafety must be demonstrated with appropriate record-keeping. The biosafety of hESC chondrodifferentiated constructs should be evaluated exhaustively in immune-deficient mice for at least six months to ''rule-out teratomas'' (Gruenloh et al., 2011), since teratomas are tell-tale signs of incomplete chondrodifferentiation processes that had left potentially dangerous pluripotent stem cells in the implant. In contrast to ESCs, adult stem cells such as MSCs have a strong safety profile in many clinical trials to-date (Griffin et al., 2010; Hare et al., 2009; Newman et al., 2009). This demonstrated biosafety makes the regulatory path for MSCs shorter, requiring instead a thorough examination of all tissues in recipient animals since MSCs often migrate to multiple tissues post-implantation. Long-term proof-of-efficacy in animal models with an absence of teratomas would move hESC-based products past the proof-of-concept stage.

Fixation and integration of stem cell-based neocartilage may rely on suturing, tissue glues, and/or cell infiltration. Loose implants are rapidly destroyed with loading, and effective integration methods must be identified during early product development. Implants must also provide mechanical function. Tissue-engineered cartilage can be immediately functional if neotissue and native tissue properties match. Temporary immobilization may be necessary for a joint receiving an implant that is expected to mature to functionality *in vivo*. Establishing a robust mode of neocartilage implantation at early stages of product development accelerates clinical translation and commercialization.

Manufacturing

The lack of sufficient FDA-compliant manufacturing sites impedes clinical translation of stem cell-based cartilage therapies. Such facilities need to operate under FDA guidance

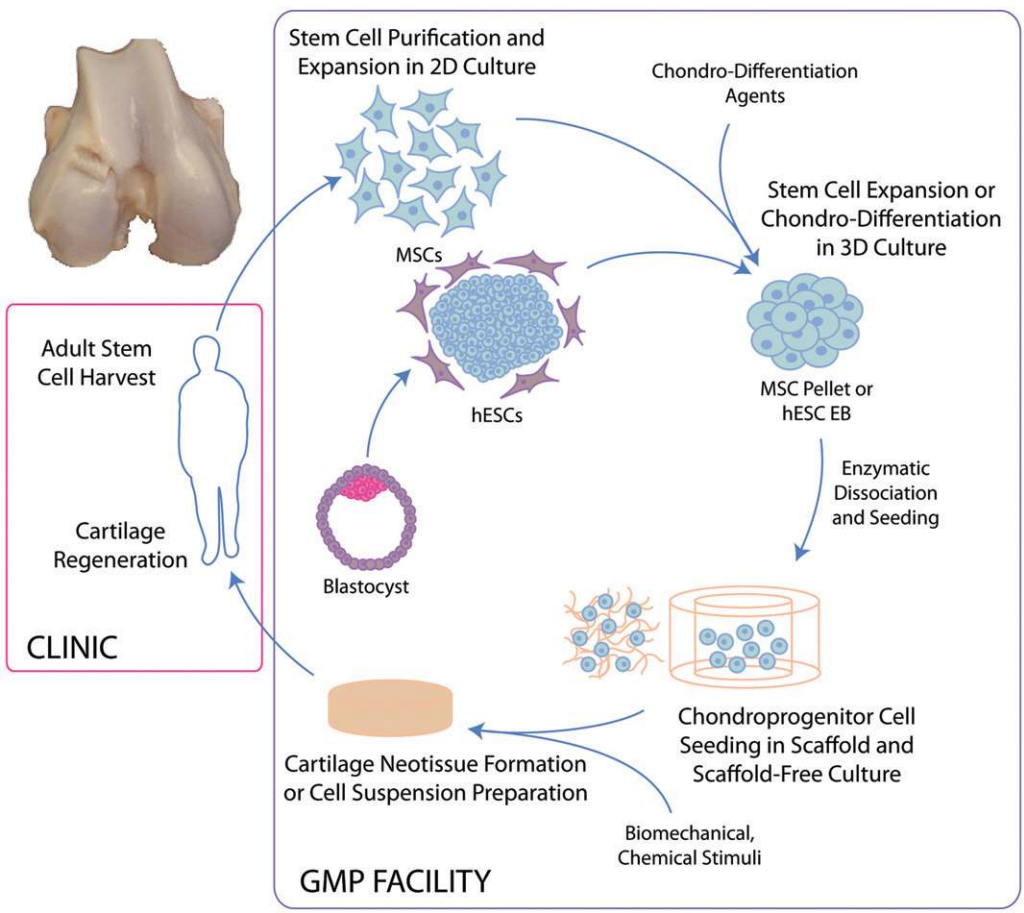

Figure 1. Overview of stem cell-based neocartilage formation. Patient-derived adult stem cells or patient-matched ESC-derived cells are sorted and expanded in 2D culture before 3D aggregate differentiation. Cells are typically dissociated and seeded into scaffolds or used in a scaffold-free approach to generate mechanically and biochemically robust neocartilage for *in vivo* implantation. For clinical translation, cellular manipulations must be performed in facilities compliant with GMP.

documents concerning product specifications, manufacture, and regulation (FDA, 2011b,c). Additionally, companies should consult the FDA on a case-by-case basis as different products' regulations and manufacturing requirements may vary. Continual communication with the FDA is an absolute necessity for establishing manufacturing sites and passing facilities inspections.

Good Tissue Practice (GTP) and other manufacturing requirements, described in the Code of Federal Regulations Title 21, Sections 1270 and 1271 (21 CFR 1270, 1271), must be followed by any facility producing human cells, tissues, and cellular- and tissue-based products (HCT/Ps). GTP compliance falls within GMP guidelines and ensures the manufacture of a sterile, efficacious and uncontaminated HCT/P. To generate products used in clinical trials or for commercialization purposes, the manufacturing facility must operate and be maintained in compliance with GTP standards. Although designing a GTP-compliant enterprise to manufacture stem cell-based cartilage is expensive, and represents a major hurdle in clinical translation, more of these establishments are needed.

Regulation of stem cell-based cartilage products

Product classification

HCT/Ps are classified under section 361 or 351 of the Public Health Service Act (PHS). Section 361 focuses on preventing the introduction, transmission and spread of communicable diseases, while section 351 regulates drugs, devices, and/or biological products. HCT/Ps are further regulated under section 21 CFR 1271. A HCT/P regulated under PHS 361 must be minimally manipulated, intended for homologous use, and uncombined with another article. Homologous use products perform the same basic function in the recipient as in the donor, e.g. hematopoietic stem cells that reconstitute the blood. Stem cell-based cartilage products will likely exceed minimally manipulation to result in mature, implantable cartilage and will most likely be regulated as a drug, device and/or biological product, falling under PHS 351.

To determine HCT/P classification as a biologic, device or combination product, the FDA provides several guidance documents (FDA, 2011d) offering definitions and examples of each type. Exact definitions for biologic, device and combination products are set forth in 21 CFR 1271 Part 3. Stem cell-loaded scaffolds, drug-eluting meshes and chemical-secreting cells all fall within the combination product category. Cartilage tissue engineering approaches using stem cells generally produce combination products. If classification of a cartilage repair or replacement HCT/P is still ambiguous given the definition, questions can be directed to the Office of Combination Products (OCP).

Regulation as a biologic, device or combination product

Stem cell-based cartilage products will likely require three tiers of testing: initial development and ''proof-of-concept'' studies, preclinical studies performed under Good Laboratory Practices (GLP), and clinical studies. Preclinical work in animal models should demonstrate safety and the biological response, durability, toxicology and dose response of the technology. The product used in animals should be nearly identical to the human product. Analogous alternatives exist, e.g. if the product uses autologous human MSCs, the animal study may employ autologous animal MSCs. Despite the large number of veterinary patients treated with stem cell-based products, their results are not necessarily usable *in lieu* of preclinical animal work.

Several FDA centers evaluate the safety and efficacy of new technologies. Potential new products, considered biologics, devices or both, are regulated by different centers that guide and oversee the approval process (Figure 2). The Center for Devices and Radiological Health (CDRH) and the Center for Biologics Evaluation and Research (CBER) are responsible for devices and biologics, respectively. Technologies with both device and biologic components (e.g. scaffold-seeded stem cells) are regulated as combination products. Regarding the guidance documents, these products are assigned to the most relevant FDA center and must conform to the regulations of both centers.

Prior to using a product for clinical trials, there must be an approved investigational device exemption (IDE) or investigational new drug (IND) application, in addition to institutional review board (IRB) approval. In general, market approval for biologics require INDs whereas devices require IDEs, with both pathways ultimately aiming to ensure product safety and effectiveness. Deciphering product classification and the relevant pathways may increase interaction with the FDA, which in turn, may facilitate the process of obtaining approval.

Several laws and published FDA documents govern the regulatory approval of stem cell products for cartilage repair. A technology using human stem cells, even if the product is de-cellularized, is a HCT/P that needs to be evaluated for purity, reproducibility and stability. The FDA considers products intended to repair knee cartilage, whether cellular or not, as significant risk devices requiring IDE and/or IND submission. Though the FDA provides a template to follow to register a product, it is still not clear whether one needs to regenerate articular cartilage in order to achieve improved patient outcome. It may be that simply repairing articular cartilage may provide a benefit tantamount to regenerating articular cartilage. The expectation at this point, however, is that by following the FDA guidance documents, not only will functional repair or regenerative neocartilage be produced, but that the tissue will also provide improved patient outcomes in terms of pain relief and restoration of joint function. Although there are FDA guidance documents and laws relevant to cartilage regeneration technologies, the approval process is product-specific and requires continual communication with the FDA.

Clinical studies are carried out in several phases to demonstrate product safety and efficacy (Figure 2). After clinical trials conclude, the product's lead center can approve the product for marketing by providing a biologics license application (BLA) or a pre-market approval (PMA) via CBER or CDRH, respectively. Post-marketing meetings with the FDA to review clinical data ensure that the product is safe and effective.

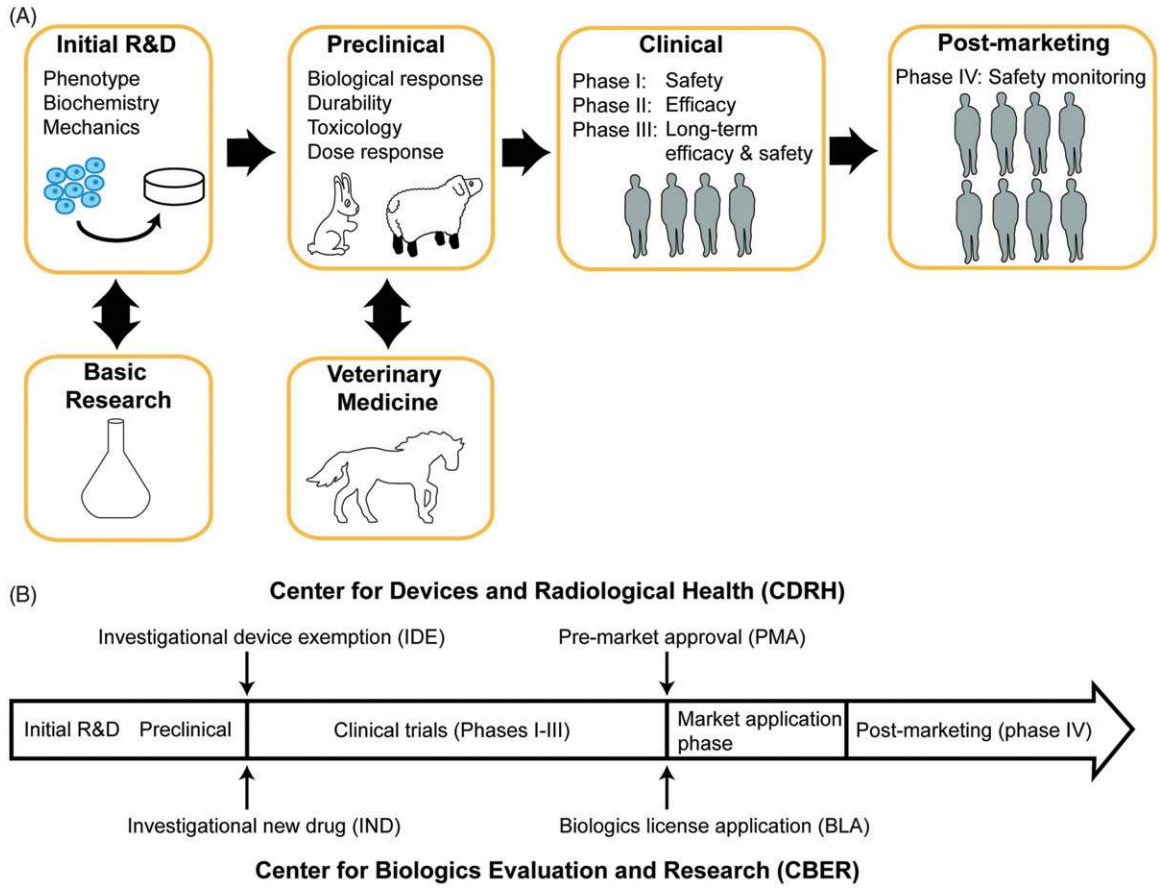

Figure 2. Translation of stem cell products for cartilage regeneration. (A) FDA regulation of biologics requires stem cell products to exhibit purity, reproducibility, and stability. Neocartilage is evaluated in preclinical trials for biological response, durability, toxicology, and dose response. Multi-phase clinical studies are used to evaluate dosage, efficacy, and safety. (B) Two major regulatory centers exist: CDRH for devices and CBER for drugs and biologics. Prior to initiating clinical trials, a product must receive an IDE or IND. After clinical trials, market approval enables clinical application of the product.

Case studies: Chondrogen, RepliCart, and Cartistem

US-based Osiris's Chondrogen illustrates the commercial development process of stem cell-based cartilage regeneration. Preclinical studies demonstrated that intra-articular MSC injection promotes regeneration following meniscectomy, prompting a Phase I/II clinical trial testing two dosages (50 or 150 million human MSCs) for safety and efficacy. Chondrogen was found to significantly improve patient pain in a dose-dependent manner, improving pain scores from 6 months to 1 year (Osiris Therapeutics, 2012).

Similarly, the Australian company Mesoblast demonstrated, in preclinical sheep studies, that osteoarthritic joints receiving RepliCart, an allogeneic MSC product, experienced significantly greater tissue thickness and ''biomechanical strength'' over control joints (Ghosh et al., 2009). RepliCart is now in clinical trials to evaluate safety at 12 months, and prevention of osteoarthritis and cartilage loss at a second time point. Chondrogen and RepliCart both mirror veterinary successes in illustrating the potential of cell suspension-based products.

A third example involves a tissue-engineered product which, unlike cell suspensions, employs a biomaterial in conjunction with stem cells. The Korean company Medipost actively markets and sells Cartistem, an umbilical cord blood-derived MSC and semi-solid polymer-based treatment, to treat arthritis and to heal cartilage injury. This allogeneic product received approval for sale and clinical use in Korea in early 2012. The FDA recently approved Phase I/IIa USA clinical trials for Cartistem. USA approval of Cartistem will pave the way for future stem cell-based, tissue-engineered products for cartilage repair and regeneration.

Future directions and conclusions

Various strategies can promote chondrogenesis of both hESCs and MSCs for regenerating cartilage. Using the expansion capabilities and flexible lineage potential of MSCs and hESCs, researchers employ 3D culture techniques, tissue engineering scaffolds, and scaffold-free differentiation methods to promote chondrogenesis. The ultimate goal of chondrodifferentiating stem cells is to provide a cell source that can be used to engineer neocartilage capable of functioning in strenuous joint environments. Success criteria based on native cartilage physiology, *in vivo* cartilage development and the associated regulatory pathways will inform future development of stem cell-based tissue engineering technologies.

Many stimuli have been used to chondrodifferentiate stem cells for tissue engineering. However, the efficiencies among studies in recapitulating native values are often not discussed. Selecting an optimal regimen for engineering neocartilage is

therefore difficult as published data are often not normalized to native tissue values. Furthermore, for statistical optimization of differentiation or tissue engineering protocols, the existence of diverse success criteria necessitates a variable that can evaluate them simultaneously. Establishment of a quantitative parameter, such as a ''functionality index'' that equally weighs biomechanical and biochemical properties normalized to native tissue (Elder & Athanasiou, 2009), will enable optimization and key comparisons of various protocols.

To create clinically applicable neocartilage, larger constructs must be formed by improving stem cell expansion and efficiency of differentiation techniques to obtain larger cell numbers. A modular approach of stem cell differentiation followed by tissue engineering of lineage-committed cells may alleviate these size considerations. In the first module, any stem cell can be chondrodifferentiated. In the second module, chondrodifferentiated cells can be used in tissue engineering to obtain robust neocartilage with clinically significant dimensions. Enhanced chondrodifferentiation and subsequent protein synthesis may result from applying optimized stimuli during each phase. By investigating the phases independently, differential effects of each regimen can be identified.

Appreciation of FDA guidelines for products, facilities, manufacturing, and regulatory processes, as outlined in guidance documents, will expedite the development of stem cell-based treatments for cartilage diseases. By enhancing dialogue with the FDA, necessary design characteristics can be integrated into early iterations of a product, speeding the time to clinical trials, with the goal of market approval and product commercialization. Integrating an understanding of the major hurdles impeding clinical translation of stem-cell based cartilage products with basic or translational research could ultimately lead to the first U.S. licensed, stem cell-based cartilage repair product.

Search strategy and selection criteria

Review content was identified via searches of PubMed, FDA.gov, and relevant articles using the search terms: ''clinical stem cell cartilage,'' ''cartilage tissue engineering'' and ''stem cell animal model cartilage''. Only articles and papers published from 1991 to 2013 in English were considered. Tables 1 and 2 were populated based on a PubMed search of ''stem cells'' AND cartilage AND X, where X is a growth factor (e.g. TGF-β1, BMP-2) or mechanical stimulus (e.g. compression, tension). With a balance between space constraints and scholarship, up to five unique laboratories per stem cell type per growth factor or mechanical stimulus have been reported during the past 10 years. By consulting the works cited in these tables, the reader can readily gather additional factors that are used by multiple groups, such as dexamethasone. To avoid redundancy, these commonly used factors have not been included in these tables.

Declaration of interest

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