Tissue engineering and its application in veterinary medicine: A review

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Abstract
Tissue engineering is an amalgam of principles and techniques of various disciplines which aims at assembling biological substitutes to aid either in growth of new tissues or to restore the structure and function of affected tissue. It involves construction of a three-dimensional matrix which mimics the natural tissue in all aspects viz, functionally, structurally and mechanically by use of four key materials (i.e., scaffold, growth factor, extracellular matrix and cells). Although various types of cells are used in tissue engineering but autologous cells (stem cells) are preferred the most. Tissue engineering is an expanding field where in efforts are being made to include all tissue and organs of humans and animal body. The added advantage of tissue engineering is that it probably has the ability to correct various incurable defects as well as replacement of any damaged structure. Therefore it can be considered as a viable therapeutic option for replacement and regeneration of tissue. Although, the results are far more promising but it demands a lot of effort in future to be a successful tool in animal medicine.

Keywords: Tissue engineering, stem cells, veterinary medicine.

Introduction
Tissue engineering is an interdisciplinary field that applies the principles and methods of bioengineering, material science, and life sciences towards the assembly of biologic substitutes that mimic the natural extracellular matrix to help guide the growth of new functional tissue in vitro or in vivo to restore, maintain and improve tissue functions following damage either by disease or traumatic processes (Knight, 2004) [20]. In 1990s, the term regenerative medicine was used interchangeably with tissue engineering. The general principles of tissue engineering involve combining living cells with a natural/synthetic support or scaffold to build a three dimensional living construct that is functionally, structurally and mechanically equal to or better than the tissue that is to be replaced (Stock, 2001) [35]. The development of such a construct involves successful interaction between four key materials i.e., scaffold, growth factors, extracellular matrix and cells (Fuchs, 2001, Shieh, 2005 and Naughton, 2002) [15, 30, 24].

Scaffolds
Scaffolds materials are three-dimensional tissue structures that guide the organization, growth and differentiation of cells. Scaffolds must be biocompatible and designed to meet both nutritional and biological needs for the specific cell population (Vats, 2003) [77]. The main properties of biocompatible scaffolds (synthetic or natural) include optimal fluid transport, delivery of bioactive molecules, molecular degradation, cell-recognizable surface chemistries, mechanical integrity and the ability to induce signal transduction (Shin, 2003) [81]. Natural biomaterials (Alginate, cellulose, chitosan, collagen, fibrinogen, hyaluronic acid, silk fibroin, glycosaminoglycans (GAGs), hydroxyapatite (HA) etc.,) used for stem cell cultivation have an advantage of being bioactive, biocompatible with similar mechanical properties as native tissue (Chung, 2008) [9].

Growth factors and Extracellular matrix
Growth factors are soluble peptides capable of binding cellular receptors and producing either a permissive or preventive cellular response toward differentiation and/or proliferation of tissue (Whitaker, 2001) [41]. Extra cellular matrix (ECM) must be capable of providing the optimal conditions for cell adhesion, growth and differentiation within the construct by creating a system capable of controlling environmental factors such as pH, temperature, oxygen tension and mechanical forces (Naughton, 2002) [24].
These conditions are determined by particular cell lines and the properties of the scaffold (Naughton, 2002) [24].

**Cells**

Finally, the development of a viable construct involves a suitable supply of cells that are ideally non immunogenic, highly proliferative, easy to harvest and have the ability to differentiate into a variety of cell types with specialized functions (Koh, 2004) [21]. Stem cells have the potential to divide and differentiate into various specialized cell types and can self-renew to produce more stem cells (Crovace, 2010) [10]. Stem cells can be divided based on their self-renewal and potency (Crovace, 2010) [10]. Self-renewal of stem cells is the ability to go through numerous cycles of cell division while maintaining the undifferentiated state while other property of stem cells is potency which is the capacity to differentiate into specialized cell types (Nourissat, 2010) [25]. Based on the potency, stem cells can be divided into totipotent stem cells (Chen, 2012) [8] which can differentiate into embryonic and extraembryonic cell types (Nourissat, 2010) [21] and have the ability to construct a complete, viable organism (Guest, 2010) [10]. Potency of these cells is highest among other stem cell types (Nourissat, 2010) [25]. The second type is the pluripotent stem cells (Okamoto, 2010) [26]. These cells are the progeny of totipotent cells and can differentiate into almost all cells (e.g. cells derived from any of the three germ layers) (Ai, 2012) [1]. The third type is the multipotent stem cells (Chen, 2012) [8] which can differentiate into a number of cells, but only those of a closely related family of cells (Zscharnack, 2010) [48]. The potency of these cells is much lower than the totipotent stem cells and lower than pluripotent stem cells. The fourth type is the oligopotent stem cells. These cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells (Yao, 2012) [45]. Finally, the fifth group is the unipotent cells (Ter, 2012) [36] and the potency of these cells is extremely low so they can produce only one cell type, their own. They have the property of self-renewal, which distinguishes them from non-stem cells (Ter, 2012) [36]. Therefore, all types of stem cells have the ability of self-renewal but their potency is different and depends on the source that they have arisen from (Zscharnack, 2010) [48].

Current approaches to tissue engineering can be stratified into substitutive, histioconductive, and histioinductive (Knight, 2004) [20]. Substitutive approaches (ex vivo) are essentially whole organ replacement, whereas histioconductive approaches (ex vivo) involve the replacement of missing or damaged parts of an organ tissue with ex-vivo constructs. In contrast, histioinductive approaches facilitate self-repair and may involve gene therapy using DNA delivery via plasmid vectors or growth factors.

**Techniques of Tissue engineering**

When cells are used for tissue engineering, a small piece of donor tissue is dissociated into individual cells. In case of in vivo tissue engineering, patient acts as a bioreactor for cell differentiation and in case of in vitro tissue engineering, bioreactor is used for cell differentiation. These cells are either implanted directly into the host (inivivo) or are expanded in culture (invitro), attached to a support matrix, and then reimplanted into the host after expansion. The source of donor tissue can be heterologous, allogegenic (same species, different individual), or autologous. Ideally, both structural and functional tissue replacements will occur with minimal complications. The most preferred cells to use are autologous cells, where a biopsy of tissue is obtained from the host, the cells are dissociated and expanded in culture, and the expanded cells are implanted into the same host. The use of autologous cells avoids rejection, and thus the deleterious side effects of immunosuppressive medications can be avoided.

**Bioreactors**

A bioreactor is a device that attempts to simulate a physiological environment in order to promote cell or tissue growth in vitro. It is used to aid in the in vitro development of new tissue by providing a better physiological environment including temperature and oxygen or carbon dioxide concentration and extend to all kinds of biological, chemical or mechanical stimuli (Plunkett, 2011) [28]. The bioreactors used for 3D cell cultures are small plastic cylindrical chambers. I with regulated internal humidity and moisture. This humidity is important to achieve maximum cell growth and function. The bioreactor uses bioactive synthetic materials such as polyethylene terephthalate membranes to surround the spheroid cells in an environment that maintains high levels of nutrients. The bioreactor chamber is part of a larger device that rotates to ensure equal cell growth in each direction across three dimensions.

**Applications of tissue engineering in veterinary medicine**

**Role of tissue engineering in tendon defects**

Tissue engineering has been introduced to improve the outcome of incorporation of the tissue engineered grafts and improve the healing processes of injured tendons. A major advancement in tendon tissue engineering is related to the scaffolds. The first step in tendon regenerative medicine is to design a suitable environment for cell migration, proliferation, remodelling and maturation (Moshiri, 2013) [22]. Therefore, there are several factors that have an impact on the effectiveness of the scaffold including the basic material of the scaffold, architecture of the scaffold, diameter and orientation of the fibres, their biological characteristics and the amount of free spaces and pore size (Shearn, 2011 and Whitlock, 2012) [29,42]. Other issues that should be considered in manufacturing a scaffold (Chen, 2009) [7] is a suitable scaffold for tendon tissue engineering i.e. it should be cytocompatible in vitro and biocompatible and biodegradable in vivo (Shearn, 2011) [20]. Unfortunately, most of the exogenous based biomaterials for tendon repair have serious limitations, such as lower capacity for inducing cell proliferation and differentiation (tenoconductivity), poor biocompatibility and remodelling potential (tenoconductivity) (Whitlock, 2012) [42]. Basic material of the scaffold can generally be divided into three major groups including biological (natural), synthetic and hybrid materials (Chen, 2009) [7]. Biological materials such as collagen, elastin, gelatin, chitosan, albumin, alginate, fibrin and chondroitin sulphate have been shown to be effective in tendon healing (Whitlock, 2007) [43]. Their toxicity is low and has some beneficial biological role after implantation in the injured area (Wotton, 2009) [44]. Mature tendons are composed of more than 90% type 1 collagen. Elastin is also present in tendons in a much less proportion (about 1%) and its major application in tissue engineering is to produce vascular scaffolds (Chen, 2009) [7]. Chitosan is a natural polysaccharide obtained from insects. There are also some nonbiodegradable biological materials such as silk and carbon fibres (Naughton, 2002) [24]. The usage of carbon fibre did not continue because of its high
toxic effect and serious inflammatory reactions. However, investigations into silk are still in progress, as that have low value in translational medicine (Chen, 2009) [7]. Synthetic materials such polyacrylactone (absorbable), polydioxanone (absorbable), polygalactin 910 (absorbable) and nylon (non-absorbable) are other options with invaluable results (Hakimi, 2012) [17]. Several types of scaffolds with different technologies have been introduced. Tendon and ligament injuries are a frequently occurring problem not only in human but also in equine athletes. Successful therapy is challenging because of high re-injury rates following conventional treatment regimes and poor regeneration capacities of tendon tissue (Dowling, 2000 and Chong, 2009)[12,8]. Treatment of tendon injuries is challenging with major limitations of pertendinous adhesions because of proliferation of fibroblasts in a haphazard fashion (Moshiri, 2011) [23]. With the result, migration of fibroblast in the defect area is reduced followed by reduction in the amount of collagen production. Continuity of the defect area in such a tendon injury may not be established (Chalmers, 2000) [5]. Tendon transplantation is the only available option when the injured tendons are having-large tendon deficits (Zhang, 2012) [87]. Mesenchymal stem cells (MSCs) represent an attractive tool for tendon tissue repair in equines and bone marrow mesenchymal cells possess the best capability of differentiating into tenocytes (Stefanis, 2009) [84].

Role of tissue engineering in bone regeneration and healing
Bone healing has its own limitations and complications. In large massive bone defects, such as osteosarcoma, gunshot fractures, severe trauma, burn, etc, proper graft both in size and quality is needed for bone transplantation; however it may not be available for such cases. Therefore, there is a need to accelerate bone healing by increasing the amount of the newly regenerated callus in the defect area. Stem cells may have a role to aid bone formation in this regard. Musculoskeletal disorders represent a major part of all cases, especially in horses and dogs which represent a high proportion of the orthopaedic case load in veterinary clinical practice and prognosis for patients suffering from musculoskeletal disorders such as tendon or joint injuries is always poor, therefore it is not surprising that they are currently taking a leading role in mesenchymal stromal cells (MSC) therapies. The focus of attention in veterinary science is currently drawn to mesenchymal stromal cells (MSC) and their potential in regenerative medicine (Walter, 2012) [40]. Several therapies utilizing MSC for animal patients are being developed and some, like the treatment of equine tendinopathies (Smith, 2003 and Smith 2008) [33, 32] or cartilage degeneration in dogs (Black, 2007 and 2008) [4, 5]. The stromal compartment of bone marrow was the first source reported to contain multipotent progenitor cells (Fortier, 1998) [13]. For this reason, bone marrow is currently the best investigated origin of MSC. Bone marrow collection from the sternum is probably favored for cell-based therapies in equine regenerative medicine. This is due to the reliable isolation success of bone marrow-derived MSC in horses following an easy preparation procedure and separation of MSC via plastic adherence and cell culture (Vidal, 2006) [38]. The major disadvantage of bone marrow-derived MSC is the invasive collection procedure associated with the risk of complications such as hemorrhage, infection, pneumothorax, or pneumopericardium(Vidal, 2007) [39]. Similar to human beings, various forms of joint disease occur, including developmental diseases (i.e. osteochondrosis), acute accidental injuries (i.e. focal cartilage defect) and chronic acquired diseases. The ultimate result is often osteoarthritis (OA), a joint disease characterized by a progressing loss of functional cartilage matrix, synovitis and variable subchondral bone reaction. Horses suffering from OA induced by experimental osteochondral fragmentation were treated with bone marrow-derived MSC or the stromal-vascular fraction from adipose tissue (Frisbie, 2009) [14]. Dogs suffering from elbow and hip joint OA were injected with the stromal-vascular cell fraction from adipose tissue and an improvement of clinical parameters was observed (Black, 2007 and 2008) [4, 3]. Therefore, tissue engineering and the use of stem cells are important in situations where bone healing is delayed, where an arthrodesis needs to be supported, or in cases where bone loss is too important to be repaired without intervention (Walter, 2012)[40].

Role of tissue engineering in cartilage healing
The incidence of cartilage injury is very high and has minimum healing capability like that of tendon (Davatchi, 2011 and Kasemkijwattana, 2011) [11, 18]. It has been suggested that MSCs therapy can increase the rate and quality of cartilage regeneration both in animals and humans (Zscharnack, 2010) [48]. Platelet rich plasma (PRP) is reported to promote collagen synthesis and cell proliferation as well as enhance cartilage repair (Moshiri, 2013) [23].

Recent advances and future prospects
Recently, skin tissue engineering is considered to be the primary treatment for epidermal and dermal construct (Whitlock, 2012) [42]. Dermal fibroblasts are obtained from neonatal foreskin, expanded in vitro, seeded onto a scaffold of polylactic or polyglycolic acid before being cultured in a bioreactor system to generate a dermal layer (Kern, 2011) [19]. A bilaminate construct is produced by coating the dermal layer with multiple layers of keratinocytes (Bianco, 2001) [3]. Complexity and specialized conducting infrastructure of the heart and low proliferative potential of cardiomyocytes is a challenge for heart tissue engineering. Promising solution is embryonic stem cell lines. Engineered heart products include biocompatible, non-biodegradable but ineffective for longterm replacement. Nonetheless, the possibility of development of an engineered heart is exemplified by the successful manufacturing of tissue-engineered valves and myocardial infarct scar remodeling (Orlic, 2001) [27]. Tissue engineering has the ability to repair various defects which are otherwise incurable and it can be used to replace any of the damaged structure. The results thus far are very promising but a lot of effort still needs to be put to make it a viable therapeutic option for replacement and regeneration of tissue or organ in animal medicine.

Conclusion
Engineered tissues have progressively expanded clinical applicability in the future because they represent a viable therapeutic option for those who require tissue replacement or regeneration. Efforts for tissue engineering are currently underway for virtually every type of tissue and organ within the human or animal body. Various engineered tissues are at different stages of development, with some already being used clinically, a few in preclinical trials, and some in the discovery stage. More recently, major advances in the areas of
stem cell biology, tissue engineering, and nuclear transfer techniques have made it possible to combine these technologies to create the comprehensive scientific field of regenerative medicine.

Reference

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