Natural Biomaterials for Veterinary Regenerative Therapy
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ABSTRACT
Natural and synthetic biomaterials play an important role in the treatment of different diseases. Current research is trying to obtain different biomaterials with optimized characteristics. Mesenchymal stem cells with biomaterial scaffolds can provide a promising strategy for tissue engineering and cellular delivery. Because these cells are key elements of cell therapy of various diseases, evaluation and use of specific substrates is highly important. Concomitant use of stem cells and biomaterial scaffolds offer a hopeful strategy for tissue engineering. In this review, we examine the applications of the different types of natural scaffolds (collagen, fibrinogen, gelatin etc.) and evaluate their use in combination with stem cells for tissue engineering applications.

INTRODUCTION
Tissue engineering is a rapidly developing area that might restore, maintain or improve tissue functions [1,2]. The major elements of tissue engineering are: integrate cells, scaffolds and biologically active molecules [3-10]. These components proceed synergistically to regulate stem cell propagation and differentiation consequently ensuring tissue regeneration [11]. Scaffolds provide the environment and space for cells [4,12,13] and the bioactive molecules are required to induce regeneration [14]. The fundamental idea of tissue engineering is to induce tissue regeneration at diseased tissues or organs with cells and their environment [15]. This requires a local environment that enables to augment the proliferation and differentiation of cells [12,15]. The minimum requirements for biomaterials refer to present excellent biocompatibility, controllable biodegradability, appropriate mechanical strength, flexibility and ability to absorb body fluids [16]. The surface morphology is also very important on the attachment of surrounding cells and tissues after implantation. Efficient modification of the scaffold surface may play a significant role in facilitating tissue engineering. Recent findings have reported that surface modification of scaffolds with nano-sized materials or combinations of several biomaterials may stimulate bioactivity, cell proliferation, tissue compatibility and controllable biodegradability [4,17].

SOURCES OF STEM CELLS
The biotechnology of harvesting and use stem cells is the major interest given by their ability to differentiate into mature somatic phenotypes. Recent interest in stem cell biology and its therapeutic potential has led to search an accessible new source of stem cells. Stem cells are defined as cells with clonogenic ability, self-renewing and differentiation capacity in one or more specialized cell lineage [18].
Stem cells can be generally classified according to the origin into embryonic and adult (postnatal) stem cells or according to their plasticity into totipotent, pluripotent, multipotent and unipotent stem cells. While adult stem cells are derived from adult body, embryonic stem cells can be harvested from embryo from the inner cell mass [10,20]. Compared with embryonic stem cells that are often surrounded by controversy, mesenchymal stem cells (MSCs) have received wider attention because they can be easily isolated from different sources [21] including the oral cavity [22].

Mesenchymal stem cells (MSCs) which can be isolated from almost all postnatal organs and tissues [23] are stromal unspecialized cells that have the ability to self-renewal through cell division and also exhibit multilineage differentiation and immunosuppressive functions [21,24,25].

Oral cavity such as dental pulp, dental follicle, dental papilla and periodontal ligament (PDL), coronal pulp, apical papilla, subepithelial layers of oral mucosa, the gingival tissues, exfoliated deciduous teeth have been identified as easily accessible sources of multipotent stem cells that could be cryopreserved and used for autogenic or allogenic cell therapy [20,21].

An important subject for the development of differentiated cells is the practical aspects of producing optimal culture conditions (substrates, cultures medium, growth factors, etc) of these cells [26] for prolonged expansion [27]. One of the major interests of regenerative therapy requires the cultivation of stem cells on different specific substrates namely degradable scaffolds to obtain all kinds of tissues through the control and guidance of their differentiation [28].

In vitro studies have shown that cartilage extracellular matrix (ECM) components (collagen, elastin, fibronectin and growth factors (BMP-2, BMP-4, FGF, IGF, VEGF etc.) regulate the differentiation of stem cells. The three-dimensional (3D) nature of ECM can greatly affect a number of associated cells [29-31] and plays a crucial role in regulating cell behavior [11]. The dynamic composition of ECM acts as a reservoir for soluble signaling molecules and mediates signals from other sources to migrating, proliferating and differentiating cells [32]. ECM of each organ is different in its composition and in its relations with the cells is unique to that organ [31].

Artificial substitutes for ECM are some 3D structures, called tissue scaffolds, which may contain natural or synthetic polymers or a mixture of both [33].

**STEM CELLS CULTIVATION ON NATURAL BIOMATERIALS**

Biomaterial-based scaffolds are the most important tool in providing a 3D environment for cells, both in culture and inside the body. The main properties of biocompatible scaffolds (synthetic or natural) consist in optimal fluid transport, delivery of bioactive molecules, material degradation, cell-recognizable surface chemistries, mechanical integrity and the ability to induce signal transduction [34,35].

Natural biomaterials used for stem cells cultivation can consist of components found in the ECM such as alginate, cellulose, chitosan, collagen, fibrinogen, hyaluronic acid, silk fibroin, glycosaminoglycans (GAGs), hydroxyapatite (HA) etc., and therefore have the advantage of being bioactive, biocompatible, and with similar mechanical properties as native tissue [36].

Ideally the scaffold must provide certain properties a) controlled degradation; b) with the potential to maintain cell viability, differentiation, and ECM production; c) with probable diffusion of nutrients and waste products; d) adherence and integration capacity e) and mechanical integrity [37].

**Collagen**

Collagen is the key constituent of the extracellular matrix characterized by exceptional biocompatibility, low antigenicity [38] increased adhesion capacity and high degree of biodegradability [36], is an ideal scaffold or carrier for tissue engineering. Collagen contains specific cell adhesion domains [36] and its derivatives isolated from bovine cartilage are a classic natural material for tissue engineering; this organic constituent provides mechanical strength in tissues [39]. Collagen membranes ensure the attachment, growth and migration of mesenchymal stem cells without influencing the expression of the stemness genes [40].

**Fibrinogen and fibrin**

Fibrinogen and fibrin glue are another class of tissue-derived natural materials that can be utilized to create three-dimensional scaffold materials, widely utilized as a biomaterial. Fibrinogen is one of the primary components of the coagulation cascade [41]. The haemostatic coagulation processes require a combination between fibrinogen and thrombin under the catalysis of calcium ions [42,43]. It is attractive as a natural scaffold because it can be made from autologous blood.

Studies in nude mice have demonstrated the fibrin glue capacity as a good biomaterial, because the degradation and polymerization time can be controlled [37,44]. Fibrin glue can also combined with other polymers like polyurethane, in order to maintain cell viability, attachment and distribution with increased expression of aggrecan and type II collagen [57,45].

**Gelatine**

Gelatine, another biocompatible substrate, derived from collagen, can be modified as photopolymerizable gelatine that can crosslink through polymerization induced by irradiation with visible light [46]. Gelatine/alginate gels can promote chondrocyte...
proliferation and expression of hyaline matrix molecules and type II collagen with increased spatial deposition of proteoglycans
[47]. Gelatin-based hydrogels are biodegradable, with high biocompatibility, and offer possibilities to introduce functional groups
and/or ligands [48].

**Silk fibroin**

Silk fibroin is composed of a filament core protein called fibroin with a glue-like coat of sericin proteins. It is a natural biomaterial
isolated from silkworm cocoons, spiders, scorpions, mites and flies. Silk it can be converted into porous scaffolds using gas
foaming or salt leaching methods [49-52] and is widely used as suture materials for surgical applications. This biomaterial shows
good biocompatibility and suitable mechanical properties. The combination with mammalian cell makes silk fibroin an attractive
material for tissue engineering [49].

**Hyaluronan or hyaluronic acid**

Hyaluronan or hyaluronic acid is an acidic glycosaminoglycan, one of the major components of the extracellular matrix and
represents an extremely attractive natural biomaterial [53]. It is present in tissues as a gel-like substance but can be chemically
modified for efficient processing into fibers, membranes, or microspheres. A modified type of hyaluronic acid is commercially
available as Hyaff® [54].

**Chitosan**

Chitosan is a biosynthetic polysaccharide derivative of chitin harvested from exoskeletons of arthropods. This polymer is
biocompatible, degraded in vivo by lysozymes, and can interact with growth factors and adhesion proteins. The biodegradable and
biocompatible capacity is similar with glycosaminoglycans often used for in vitro studies [55-59].

**Matrigel™**

Matrigel™, a product currently available commercially, is comprised of a variety of ECM components including laminin,
collagen IV, and heparan sulfate proteoglycans [53,54] and has been used extensively in cell culture [54,56]. Is an ECM-mimicking
hydrogel produced by mouse Engelbreth-Holm-Swarm tumors. It closely resembles the native ECM with a similar composition
and assembling structure [55]. The cell microenvironment plays a significant role in determining progenitor cell fate and function.
Therefore the accurate coordination of interpreting spatial and temporal cues from their microenvironment is really essential for
stem cells to generate complex, functional tissues [60].

**CONCLUSION**

New biomaterials are being continuously developed. Their interaction with inserted cells and growth factors has a decisive
role for regenerative medicine. Understanding the complex mechanisms involved in stem cells adhesion and division allows us to
obtain useful biodegradable biomaterials for cell therapy. Successful combination of these scaffolds lead to mimicking cellular
microenvironment and maintaining pluripotent ability of these cells.

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