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Review

Fibrous protein-based hydrogels for cell encapsulation

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ABSTRACT

Tissue scaffolds play a vital role in tissue engineering by providing a native tissue-mimicking environment for cells, with the aim to promote cell proliferation, proper cell differentiation, and regeneration. To better mimic the microenvironment of native tissues, novel techniques and materials have emerged in recent years. Among them, hydrogels formed from self-assembled biopolymer networks are particularly interesting. This paper reviews the fabrication and use of fibrous protein-based hydrogels, with an emphasis on silk, keratin elastin and resilin proteins. Hydrogels formed by these proteins show close structural, chemical and mechanical similarities with the extracellular matrix, typically good biological compatibility, and they can trigger specific cellular responses. In addition, these hydrogels can be degraded in the body by proteolytic enzymes. For these reasons, fibrous protein hydrogels are one of the most versatile materials for tissue engineering.

1. Introduction

Tissue engineering aims to restore or regenerate damaged tissue by combining cells derived from a patient biopsy with engineered biomaterial scaffolds that provide a temporary extracellular matrix for the cells to attach and to proliferate [1]. At the same time, these scaffolds may also serve as carriers for growth factors, other enzymes, or drugs [1–3]. Two main strategies for combining cells with biomaterial scaffolds can be distinguished: cells are either seeded onto prefabricated porous scaffolds, or cells are encapsulated during scaffold formation [4,5] (Fig. 1). In both cases, tissue engineering aims to replicate the anatomical structure and function of the specific tissue or organ to be replaced or repaired [6]. To do so, different types of materials have been develop or used, including foam structures, microsphere scaffold, hydrogels, fibrous structures, and polymer-bioceramic composite scaffold.

In recent years, fibrous protein-based hydrogels have become popular [7,8] due to their structural and mechanical similarity with the native extracellular matrix (ECM) and their relatively simple processability under mild, cell-compatible conditions [9]. These hydrogels consist of networks of hydrophilic biopolymers that have the ability to bind large quantities of water, which together with osmotic forces prevents the network from collapsing [10]. The 3-dimensional structure of the biopolymer networks is stabilized either by chemical crosslinking (covalent and ionic) or physical crosslinking (entanglements, crystallites, and hydrogen bonds). Hydrogels are attractive as a cell matrix and as connective tissue substitutes due to their ability to form mechanically stable, porous, hydrated 3D polymer networks [11–13] that facilitate the transport of nutrients and metabolic waste products [10,14–18]. Moreover, hydrogels can be formed *in vivo* and are therefore compatible with minimally invasive surgery methods: a liquid precursor solution together with suspended cells can be injected at the site of interest, and the polymerization process leading to the hydrogel formation takes place in the body [10,18–22].

Nature offers an abundance of structural building blocks for hydrogel fabrication that can be derived from mechanically stable protein biopolymers: silk fibroin from spider webs; collagen from skin, bone and tendons; keratin from wool or hair; elastin from elastic tissues; fibrin from blood clots; resilin from insect tendons. Each of these biological materials shows unique properties unmatched by known technical materials.

The present paper will give an overview of the basic fabrication principles and properties of biopolymer-based hydrogels for tissue engineering. In particular silk fibroin, keratin, elastin and resilin will be explored in detail. However, we will not discuss collagen and fibrin here as several excellent reviews are already available [9,23–34].

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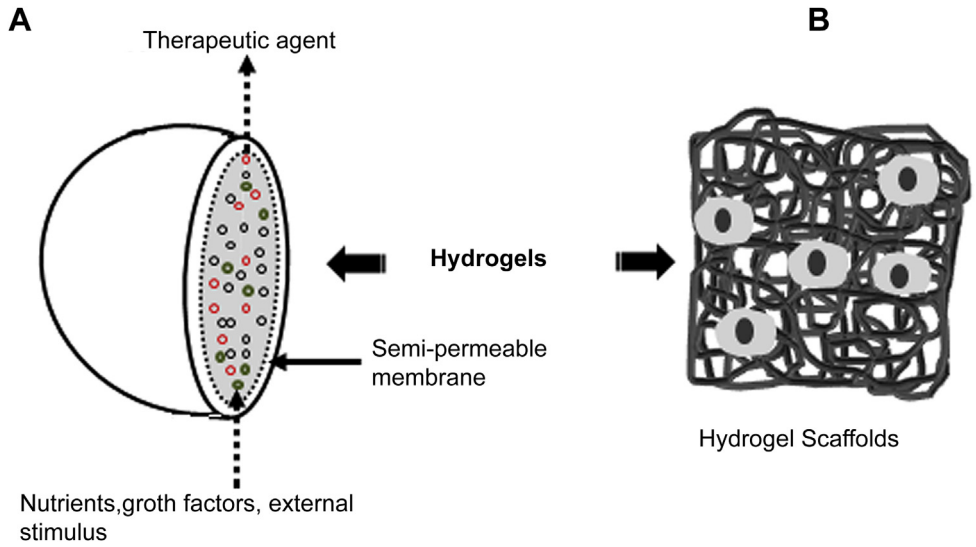

Fig. 1. Hydrogels and tissue engineering. Schematic diagram of the use of hydrogels in microencapsulation (A) and in tissue-engineering scaffold (B).

2. Biopolymer-gels based on fibrous proteins: general considerations

A wide range of natural materials can form non-cytotoxic polymeric hydrogels [21,35]. These natural polymers can be classified into proteins (i.e., silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, and myosin), polysaccharides (i.e., cellulose, amylose, dextran, chitin, and glycosaminoglycan's), or polynucleotides (i.e., DNA, RNA) [36]. In particular protein-based hydrogels can mimic features of the extracellular matrix and thus have the potential to promote the migration, growth and organization of cells during tissue regeneration and wound healing. Protein-based hydrogels are therefore also often suitable materials for cell encapsulation [37–39].

Fibrous proteins, such as collagens, elastins, silks, and keratins are characterized by highly repetitive amino acid sequences that give these proteins unique mechanical and architectural properties. These repetitive amino acid sequences result in the formation of relatively homogeneous secondary structures (e.g. β-pleated sheets, coiled coils, or triple helices), which in turn promote the spontaneous polymerization of protein monomers that self-assemble into structurally interesting hierarchical materials [40]. Furthermore, fibrous proteins are attractive materials for designing bioactive scaffolds, because cells can recognize and bind to specific sites within proteins, as well as secrete enzymes that may degrade specific amino acid sequences [41]. Table 1 summarizes the use of protein-based hydrogel materials for tissue engineering applications and as cell culture scaffolds.

3. Silk fibroin

Silks are naturally occurring protein polymers that can be found in a wide diversity of insects and spiders. The most widely used and characterized silks are from the domesticated silkworm (*Bombyx mori*) and from some spiders (*Nephila clavipes* and *Araneus diadematus*) [42,43]. Silk proteins are usually produced within

Table 1
Protein-based hydrogels and applications.

Protein	Tissue engineering application	Encapsulated/seeded cell types	Animal model	References
Silk fibroin (SF)	Bone regeneration	Osteoblasts (MG63)	Rabbit distal femurs	[89,93]
	Bone/cartilage	Human bone marrow derived mesenchymal cells	–	[91,92]
	Bone regeneration	–	Rabbits with 6 maxillary sinuses	[94]
	Bone regeneration	Human peripheral blood mononuclear cells	–	[95,96]
Keratin	Wound healing/tissue regeneration	Microvascular endothelial cells and keratinocytes	Rats with wounded on either side of the dorsal midline	[154,155]
	Regeneration of peripheral nerves	Schwann cells	Mice and rats with peripheral nerve injury or critical size nerve defect	[156,158]
	Soft tissue regeneration	L929 murine fibroblasts	–	[160]
	Regeneration of sciatic nerve injury	Schwann cells	Rats with sciatic nerve injury	[159]
	Wound healing/tissue regeneration	L929 murine fibroblasts and vascular smooth muscle cells	–	[261]
	Parkinson's disease	Hepatocytes neurospheres forming cells		[163]
Elastin	Elastic tissue	M1 murine epithelial cells and human fibrosarcoma cells	Guinea pigs	[207]
	Vascular tissue	Porcine vascular smooth muscle cells	–	[216]
	Cartilage	NIH-3T3 fibroblasts	–	[219]
	Topical/dermal application	Human skin fibroblasts	Sprague–Dawley rats	[220]
Resilin	Vocal cord	NHT-3T3 fibroblast	–	[255]
	Cartilage	Human mesenchymal stem cells	–	[257]
	Cartilage	Primary human mesenchymal cells from bone marrow	–	[258]
	Cardiovascular tissue	Human aortic adventitial fibroblasts	–	[259]

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