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Fabrication of keratin-silica hydrogel for biomedical applications

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ABSTRACT

In the recent past, keratin has been fabricated into different forms of biomaterials like scaffold, gel, sponge, film etc. In lieu of the myriad advantages of the hydrogels for biomedical applications, a keratin-silica hydrogel was fabricated using tetraethyl orthosilicate (TEOS). Textural analysis shed light on the physical properties of the fabricated hydrogel, inturn enabling the optimization of the hydrogel. The optimized keratin-silica hydrogel was found to exhibit instant springiness, optimum hardness, with ease of spreadability. Moreover, the hydrogel showed excellent swelling with highly porous microarchitecture. MTT assay and DAPI staining revealed that keratin-silica hydrogel was biocompatible with fibroblast cells. Collectively, these properties make the fabricated keratin-silica hydrogel, a suitable dressing material for biomedical applications.

1. Introduction

Hydrogels are one of the most established biomaterials because of their close resemblance to the in vivo histo-architecture of the natural extracellular matrix (ECM) [1,2]. Hydrogels are crosslinked 3D networks comprising of either natural or synthetic polymers. It has been observed that synthetic hydrogels have more controllable physical properties and are more reproducible, but show poor biocompatibility, lack of bioactivity and little resemblance to the natural environment [3–6]. Hence use of biopolymer such as collagen or keratin in the fabricated biomaterial enhances the biocompatibility and regeneration of normal at the site of applications [7–9].

Sol–gel technique is well-known and practiced to synthesize novel hybrid biocompatible gels for a wide range of applications [10,11]. It is interesting to note that sol–gel process is in-expensive and the gels so produced are non-toxic [12,13], involving simultaneous hydrolysis and condensation of alkoxide or salt. The sol–gel processes have been utilized to produce bioactive coatings, powders, and substrates that are suitable to be used as implants and sensors [14,15]. Advantage of employing sol–gel method to produce hybrid materials is that it combines both inorganic and organic properties. In addition, this method offers the possibility of obtaining homogeneous hybrid materials under low temperature, which further provides the scope of incorporating a variety of compounds [16,17].

Hydrogels have shown promising applications in the field of pharmacy and medicine, apart from their popular use in the non-medical sector [18]. This can partially be attributed to the absorption capacity and retention of mechanical strength of these hydrogels. Hydrogels are preferred because they have high water content, with soft and rubbery surface, mimicking human tissue viz., muscles, tendons and cartilage [19,20]. These characteristic features make them potential candidate for transdermal drug delivery systems. The hydrogels liquefy necrotic tissue on wound surface and prevents loss of body fluids. Moreover they are non-adherent and the dressing can be removed without trauma to the wound bed [21]. The feasibility of using natural materials including polysaccharides and proteins for the fabrication of hydrogels has been well-demonstrated. Among these materials, collagen, chitosan, keratin, gelatin, elastin, fibrin hyaluronic acid etc. exhibit prominent bioactivity in biomedical applications [22–24].

Keratins are naturally derived proteins that can be fabricated into several biomaterial forms including hydrogels [25]. Although keratins are considered to be intracellular cytoskeleton proteins belonging to the family of intermediate filaments [26], they contain the cell adhesion motifs LDV (leu-asp-val) and RGD (arg-gly-asp) [27], enabling cell attachment and inturn supporting cell growth and development [28]. Keratins in the form of coatings, fibres and films have also shown promising cytocompatibility (Reichl 2009; Rouse and Van Dyke 2010). Keratin based hydrogels have been reported to be neuro-inductive and capable of facilitating regeneration in a peripheral nerve injury [29]. Apart from this, keratin hydrogels are known to enhance wound healing by the activation of keratinocytes in the wound bed. These hydrogels also acts as a protective barrier, absorb excess wound exudates, and maintain a moist environment, which in turn helps in pain reduction [30].

In particular, silica-based hydrogels are well recognized as suitable matrices for biomaterial development. Researchers have extensively worked on sol–gel processed silica and found their potential as drug

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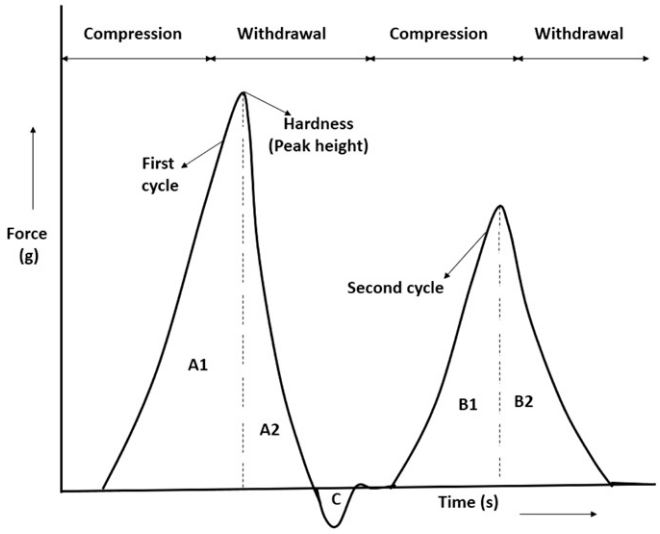

Fig. 1. Schematic representation of texture analysis to calculate i) Hardness (peak height), ii) Cohesiveness (B1 + B2)/(A1 + A2), iii) Adhesiveness (C), iv) Compressive strength (peak stress/peak strain), v) Resilience (A2/A1).

carriers [31–33]. It has been established that sol–gel produced pure silica are biocompatible and even enhance the formation of fibroblast or osteoblast leading to increased collagen production [12,34–36]. Our group has established the use of sol–gel processed collagen – silica composite materials for wound healing applications [37,38]. Varying the amount of silica could tune the mechanical property of the collagen scaffolds obtained [39].

Gorji, Allahgholi Ghasri, Fazaeli and Niksirat [13] used tetraethyl orthosilicate (TEOS) as a precursor for preparation of silica gels. It is a well-known fact that the hydrolysing agent in the TEOS-derived sols is water. Usually acid hydrolysis of TEOS is employed to prepare aggregative stable spinnable sols. Inorganic acids like hydrochloric acid (HCl), nitric acid (HNO_3), etc, are frequently used as acid catalysts, but occasionally organic acids like acetic acid is also being used [10,40,41]. There are two different ways through which sol–gel synthesis can be carried out i.e. single-stage (acid) or two-stage (acid/acid) hydrolysis of TEOS [42–44]. In both the cases, first stage is often carried out with scarcity of water. TEOS acts as a crosslinking agent for keratin hydrogel formation. TEOS has a remarkable property to produce silicon dioxide (SiO_2) when mixed with water in presence of a catalyst which can be an acid or a base as per Stober reaction which was discovered by Werner Stober. This process takes place via LaMer model or nucleation model comprising of two steps. It begins with nucleation (fast process) followed by a slow process of particle growth. In a typical process, silicon

Table 1
Gelation time of keratin-silica hydrogel at varying concentration of silica.

Ratio of silica:keratin (*w*/*w*)	Gelation time
0.4:1	No gelation
0.8:1	No gelation
1.2:1	2 h
1.6:1	1 h 30 min
2:1	1 h 10 min
2.4:1	1 h
2.8:1	40 min
3.2:1	20 min
3.6:1	15 min
4:1	10 min
4.4:1	8 min
4.8:1	7 min
5.2:1	6 min
5.6:1	5 min

Fig. 2. Representation of keratin-silica hydrogel withstanding tube inversion test (4:1w/w of silica:keratin).

alkoxide viz., tetraethyl orthosilicate (TEOS) is hydrolyzed by water in presence of an acidic catalyst. These reactive silanol (Si–OH) groups of the hydrolyzed silicon alkoxide go through condensation reactions which depend on the pH and temperature of the solution, to form siloxane (Si–O–Si) bonds, producing a 3D porous gel structure [45].

Earlier we have established that keratin can be effective for proliferation of fibroblast cells [7]. Moreover, different forms of keratin based materials are required as effective materials for soft tissue engineering applications such as wound healing. This paper deals with the development of a novel keratin hydrogel system by combining keratin with silica, where the sol–gel transition of silica had been advantageously used for the establishment of hydrogel.

2. Materials and methods

2.1. Materials

Keratin was extracted from bovine hooves, TEOS ($C_8H_{20}O_4Si$) was obtained from Alfa Aesar (United States), HCl was obtained from Fisher

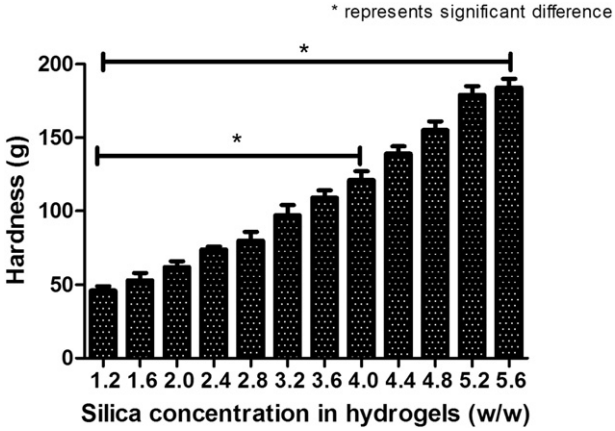

Fig. 3. Hardness of keratin-silica hydrogels with varying concentrations of silica.