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# Semi-degradable porous poly (vinyl alcohol) hydrogel scaffold for cartilage repair: Evaluation of the initial and cell-cultured tribological properties

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ABSTRACT

Tissue engineering for articular cartilage repair has shown success in ensuring the integration of neocartilage with surrounding tissue, but the rapid restoration of biomechanical and biotribological functions remains a significant challenge. Poly (vinyl alcohol) (PVA) hydrogel is regarded as a potential articular cartilage replacement for its fair mechanical strength and low surface friction, while its lack of bioactivity limits its utility. Combining the advantages of tissue engineering materials and PVA hydrogel, we developed a semi-degradable porous PVA hydrogel through addition of ploy (lactic-co-glycolic acid) (PLGA) microspheres and salt-leaching technique. Friction coefficient, continuous friction tested by a ball-plate tribometer and worn surface observed by Environmental Scanning Electron Microscopy (ESEM) were characterized for scaffolds prepared with variable porogen and PLGA content. Scaffolds cultured with rabbit chondrocytes for 4 weeks were also studied. The results showed that friction coefficient increased with a rise in porogen content, while it firstly increased and then decreased with increasing PLGA content. Similar results were obtained from cell-cultured scaffolds. In continuous friction test and worn morphology characterization, samples were more prone to be damaged with an increase in porogen and PLGA content. However, wear resistance was obviously improved for all scaffolds after 4 weeks of culture, though friction coefficient went up to a certain extent.

## 1. Introduction

Articular cartilage is a natural tissue at the ends of the bones in synovial joints, which normally performs the characteristics of low-friction, wear resistance and load bearing (Kurosawa et al., 1980; Simon, 1971; Wright and Dowson, 1976). However, as the limited capacity for self-repair, patients diagnosed with the articular defect are at the risk of tending to early osteoarthritis (Mankin, 1982; Newman, 1998).

To successfully treat with articular defect, tissue engineering is widely regarded as a potential approach and has become an attractive investigation, aiming to repair or regenerate cartilage tissue by combination of designed biodegradable scaffold, implanted cell and biologically active molecules. Scaffolds, constructed with polymers such as chitosan, poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their co-polymers (PLGA), are potential matrices applied in tissue engineering for cartilage repair, which perform excellent cell adhesion and proliferation (Chu et al., 1995; Kim et al., 2003; Lohmann et al., 2000; Uematsu et al., 2005). Moreover, the regenerative tissue obtained by this method is well integrated with the surrounding native tissue (Han et al., 2008; Uematsu et al., 2005). Even so, there is a significant drawback that the renewed tissue, named fibro cartilage owning inferior mechanical properties, is virtually different from the natural hyaline cartilage (Han et al., 2008; Solchaga et al., 2005). On the other hand, appropriate initial properties involving biotribology and biomechanics before implantation are undeniable challenges for ideal scaffolds (Guilak et al., 2001; Martens et al., 2003).

In view of the insufficiency of initial properties referred to the tissue engineering biomaterials, PVA hydrogels are proposed as replacement for articular cartilage due to their good performance characteristics, such as their chemical stability, biocompatibility, mechanical properties, or low friction coefficient (Bray and Merrill, 1973; Gu et al., 1998; Noguchi et al., 1991; Pan et al., 2007). Furthermore, the three-dimensional structure of PVA hydrogel, acquired through irradiation, chemical reaction and freezing-thawing circles, is similar to the highly nanoporous structure of natural cartilage (Hassan and Peppas, 2000). However, the lack of bioactivity leads to incompatibility to support cell adhesion and thriving on the PVA hydrogel as matrices, thus failing to integrate with the native cartilage (Maher et al., 2007).

More recently, a few researchers have focused on the combination of degradable tissue engineering biomaterials and PVA hydrogel, manufacturing the “semi-degradable scaffold” which has been found

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that chondrocytes are successfully adhesive on the scaffold and immature cartilage tissue forms after vitro culture (Scholten et al., 2011; Spiller et al., 2011). Furthermore, the mechanical property of the semi-degradable scaffold observed is similar to the healthy cartilage (Spiller et al., 2011). Nevertheless, the biotribology, as a significant property of cartilage replacement, has never been paid much attention on.

In this contribution, to combine the advantageous properties of PVA hydrogel and PLGA while diminish their disadvantages, we developed a semi-degradable porous PVA hydrogel through addition of PLGA microspheres and salt-leaching technique. Porogen and PLGA content, as two key factors impacting the tribological properties of semi-degradable scaffolds, were systematically investigated. Our hypothesis is that the semi-degradable scaffolds initially possess some certain biotribological properties and further exhibit favorable tribological performance after co-culture with chondrocytes. Furthermore, the tribological properties of chondrocyte-seeded scaffolds cultured *in vitro* for a certain period, were estimated to obtain a comprehensive understanding of the potential of the semi-degradable scaffolds as suitable cartilage replacement.

## 2. Materials and methods

### 2.1. Materials

PLGA (50:50 lactide to glycolide ratio, $M_w$=65000) was purchased from Jinan Daigang Biomaterial Co., Ltd, China. PVA (99+% hydrolysed, $M_w$=89000–98000 and 1788 low-viscosity) were separately obtained from Sigma-Aldrich, St. Lousis, Mo, USA and Aladdin-reagent Co., Ltd, China. Poly(vinyl pyrrolidone) (PVP, K-30), dichloromethane, acetone, sodium chloride powder, silver nitrate and physiological saline solution were all procured from Sinopharm Chemical Reagent Co., Ltd, China. Octaphenyl Polyoxyethyiene (OP) emulsifier was purchased from Shanghai Jiuyi Chemical Reagent Co., Ltd, China. Bovine serum was gained from Nanjing Bioon Bio-Technology Co., Ltd, China. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and Trypsin-EDTA solution (0.05% trypsin and 0.02% EDTA) were purchased from Gibco Invitrogen, Carlsbad, CA, USA.

### 2.2. Scaffold fabrication

The whole procedure of fabrication includes two steps (schematically outlined in Fig. 1). In step 1, PLGA microspheres were prepared using a modification of previously described method of emulsion-evaporation technique (Borden et al., 2002). Briefly, 480 mg PLGA was dissolved in a mixture solution of 10 mL dichloromethane and 5 mL acetone, and the solution was added dropwise to the 160 mL PVA solution (2% w/w, 1788 low-viscosity) stirred at a speed of 1000 r/min by magnetic stirrer (IKA Big Squid). After the addition, the solution was transferred to ice-water bath and vigorously stirred for 10 min to obtain a homogeneous suspension, which was subsequently stirred at a 400 r/min speed for 12 h to evaporate dichloromethane and acetone completely. The resultant PLGA microspheres were obtained through centrifugation and lyophilization. The size distribution of PLGA microsphere was measured with Malvern laser particle size analyzer (MS2000) and the result is represented in Fig. 2. It can be seen that the size range was from 0.47 μm to 40.93 μm and the majority of microsphere size was around 15 μm. In step 2, the porous semi-degradable scaffold was fabricated based on a salt-leaching technique and PLGA microspheres as an addition were appended to the composite porogen. The process in brief goes as follows: NaCl powder, milled to randomly shaped microparticles with size between 150 μm and 250 μm, together with PLGA microspheres were poured into the OP emulsifier (weight ratio between NaCl and OP emulsifier was 1:2) and stirred to form a uniform suspension. The mixture was then slowly poured into 15 wt% PVA/PVP (weight ratio is 99:1) solution at stirring speed of 500 r/min and maintained stirring for 2 h after completing adding. The resulting solution was poured into customized Poly (methyl methacrylate) (PMMA) mould (a designed shape of disk with size of 4 mm in height and 32 mm in diameter) and subjected to 7 freezing-thawing cycles (the number of cycle was designed to be 7 because the properties of PVA hydrogel nearly change after 5 freezing-thawing cycles), consisting of 21 h at −20 ℃ and followed by 3 h at 25 ℃. The resultant hydrogels were taken out from the mould and washed in distilled water with thermostatic ultrasonic equipment. The water was replaced every 1 h and the NaCl content was checked by dropping silver nitrate solution into washed water. The salt-leaching process was continued until no precipitate by silver nitrate was observed. Finally, the porous semi-degradable scaffold was acquired.

In present study, the content of PLGA and composite porogen (NaCl and OP) were two variable parameters in this study. all the specimens were labeled as “A/B”, in which A standed for the relative content of composite porogen to PVA/PVP solution, and B standed for the relative content of PLGA microspheres to dry PVA and PVP powder. In detail, the content of PLGA was held at a constant value of 20% and that of composite porogen varied from 10% to 25% (specimens labeled with 10/20, 15/20, 20/20, 25/20, separately). Furthermore, the content of composite porogen was kept at a constant value of 20% and PLGA content varied from 0% to 45% (specimens labeled with 20/0, 20/15, 20/30, 20/45, separately). Totally, eight different kinds of samples were prepared for subsequent study. Samples prepared for cell culture were sterilized in 75% (v/v) medicinal alcohol for 2 hours.

### 2.3. Cell isolation and in vitro culture

Chondrocytes were isolated from the articular surface of a mature male New Zealand white rabbit (4 months old, 2.5 kg) under sterile conditions as described by Iwasaki et al. (2004) and expanded in culture medium containing 90% DMEM, 10% FBS, 50 mg/mL ascorbic acid, 100 U/mL penicillin and 100 μg/mL streptomycin, at 37 ℃ in humidified incubator containing 5% $CO_2$. The medium was changed every 2 days. When the adherent cells reached subconfluence, they were passaged after trypsinization (0.05% trypsin and 0.02% EDTA). The second-passage cells were harvested by trypsinization followed by addition of fresh culture medium to create a new suspension with a concentration of $2\times10^7$ cells/mL. Hydrogel samples prepared with different porogen and PLGA content were inoculated in 6-well flat-bottom culture plates. 20 μL cell suspension was added to each sample located at the center of the well. The cell seeded hydrogel constructs were cultured at 37 ℃ in 5% $CO_2$ and medium were changed every 3 days. All the samples were cultured for 4 weeks before further study.

### 2.4. Scanning electron microscopy (SEM)

The morphology of PVA/PLGA semi-degradable scaffolds prepared with various composite porogen and PLGA were observed by JSM-6380LV scanning electron microscopy at 30 kV. All specimens were dried by lyophilizer for 48 hours prior to SEM observation.

### 2.5. Friction test

The equipment used in present experiment was a ball-on-plate home-built tribometer as previously described (Pan and Xiong, 2009). The scaffold sample was fixed on the rotating part of the tribometer and lubricant (physiological saline or bovine serum) was put on the surface of the sample. The friction tests were run at room temperature. When the scaffold sample was rotating against the stainless steel ball (12 mm in diameter; surface roughness is 0.1 ± 0.05 μm), the normal 5 N load was applied by poise (pressure at the interface was theoretically calculated to approximate 0. 44 MPa according to the Hertzian contact assumption) and rotating speed of sample was controlled by frequency