Non-Degradable Porous Poly(Vinyl Alcohol) Hydrogels for Cartilage Tissue Engineering

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Introduction: Defective cartilage tissue is a major clinical problem. To date, there have been no successful strategies to repair or regenerate cartilaginous tissues with successful long-term outcomes. Current strategies employ degradable polymeric scaffolds to deliver chondrocytes which often suffer from lack of strength of the construct, as well as incomplete matrix production. We propose to use a non-degradable porous hydrogel scaffold that can provide necessary and tailored mechanical strength to cartilage repair constructs seeded with chondrogeneic cells. This will allow natural dynamic load transfer to the cells, which has been shown to be a major part of their developmental pathway to produce the correct composition of cartilage. Using theta gelation method in the presence of two competing gelling agents; a high molecular weight polymer such as polyacrylamide (PAAm) and a low molecular weight polymer such as Polyethylene glycol (PEG), we developed a porous polyvinyl alcohol (PVA) hydrogel with interconnected pores and continuous channels (Fig 1-2) large enough to allow chondrocyte infiltration and extracellular matrix (ECM) deposition (1). The effect of molecular weight (MW) and concentration of gelling agent on physical properties of the hydrogel is reported in this study. These hydrogels enhanced the neocartilage formation in a subcutaneous nude mouse model using swine articular chondrocytes (2).

Materials and methods: Porous PVA hydrogels were prepared by dissolving PVA (MW=115,000g/mol) in the presence of (1) Polyacrylamide-co-Acrylic acid (PAAm-co-AAc, PVA-A) and (2) PAAm in DI water at 90°C. Solutions (1) and (2) were separately mixed with the gelling agent consisting of two different molecular weight PEG (MW=400g/mol and PEG600g/mol) to make gels PVA-A and PVA-B, respectively. A third gel was made with PVA and PAAm and mixed with PEG400/PEG200 mixture (PVA-C). Each solution was molded and cooled to room temperature for gelation for 24h [1]. Gels were rehydrated in DI water at room temperature until equilibrium. ***The equilibrium water content (EWC)*** was measured using a Thermogravimetric analyzer. ***Total creep strain (TCS)*** was determined by applying a 100N load for 10 hours followed by a relaxation period under a 10N load for 10 hours on cylindrical samples in DI water at 40°C. A phenomenological model was developed to quantify the viscoelastic response of hydrogels and cartilage to stresses. The model used here was an extension of Kelvin-Voigt model [4]. To estimate the parameters we fitted our model to experimental results and minimized the mean squared error. ***Porosity*** of the hydrogels was measured with solvent extraction method using ethanol. Pore size was determined by using the Image Analysis Software [5]. PVA-A hydrogel was seeded with swine articular chondrocytes (PVA-H) and implanted in nude mice for 6 weeks. Upon retrieval the construct was tested for EWC and creep.

Results: Both PVA-PAAm-co-AAc and PVA-PAAm formulations had open pores (20-200µm) and interconnecting channels (Fig 1). Changing the high molecular weight gelling agent from PAAm-co-AAc to PAAm did not change the overall porous structure of the hydrogel significantly (Fig 1a, 1b, Table 1). Replacing lower molecular weight gelling agent mixture PEG400/PEG600, with PEG400/PEG200 resulted in smaller pores; average pores size of PVA-C (100µm) was smaller than PVA-A and PVA-B (180 and 160µm) (Fig 1, Table 1). EWC of all three hydrogels, PVA-A, PVA-B, and PVA-C (90%), and the implanted hybrid construct (91%) was higher than EWC of swine cartilage (77%). All three hydrogel types showed similar unconfined creep resistance (~80%) to that measured with swine cartilage (81%). Ex vivo the hydrogel/cell construct (PVA-H) exhibited better creep resistance than the acellular hydrogels and swine cartilage. Elastic modulus of the acellular hydrogels was higher than those of swine cartilage and PVA-H. Time dependent response to creep deformation (T) of all acellular hydrogels, PVA-H, and swine cartilage were similar (Table 1). After the *in vivo* period the PVA-H stained positive for the presence of proteoglycans. There was no fibrous tissue at the interface between the hydrogel matrix and the cartilage tissue (Fig 2).

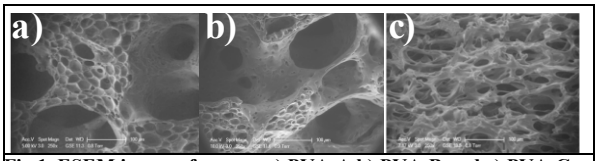

Fig 1. ESEM image of porous a) PVA-A b) PVA-B and c) PVA-C

Table 1 EWC, TCS, elastic modulus (EM), time constant (T), porosity(ϕ), and average pore size (APS) of the PVA hydrogels

Sample	EWC (%)	TCS (%)	EM (MPa)	T (h)	Φ (%)	APS (mm)
PVA-A	90±0.3	83±4	4±2	1±0.6	82±5	180
PVA-B	91±0.4	81±1	4±0.1	2±0.3	85±5	160
PVA-C	90±0.4	80±1	4±0.2	2±0.1	84±5	100
PVA-H	91±1	74±2	2±0.1	2±0.02	-	-
Swine Cartilage	77±0.3	81±4	2±0.1	2±0.3	-	-

Discussion: Pore size and the morphology of the hydrogels are not only important to host the chondrocytes, but they also play a key role to maintain nutrient flow. When prepared by theta-gelation method, PVA hydrogels exhibit semi-crystalline gel networks with 10-20µm closed pores (1, 3). In theta-gel method, addition of a low molecular weight gelling agent PEG into aqueous PVA solution reduces the quality of the solvent with decreasing temperature, forcing the PVA to phase separate and crystallize. One can alter the pore morphology of the gel network by changing the molecular weight and concentration of PVA and the gelling agent. We modified the theta gelation method by using high molecular weight gelling agent (a non-ionic (PAAm) or an ionic (PAAm-co-AAc)) along with PEG in PVA solution which resulted in larger pores (50-200µm). We also found that the pores were interconnected and open to the surface which is desirable to grow cartilage. The porous PVA hydrogels in their acellular form and after cell seeding and 6 weeks in vivo were similar to swine articular cartilage in terms of their viscoelastic properties.

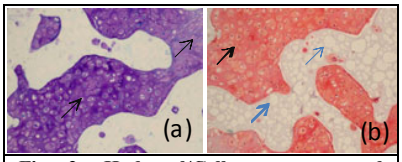

Fig 2. Hydrogel/Cell construct and cartilaginous matrix after 6 weeks in nude mice stained with toluidine blue (a) and Safranin-O (b).

Significance: Degradable polymeric scaffolds currently applied in tissue engineering to deliver cells in situ for cartilage repair suffer from weak constructs and incomplete matrix production. Non-degradable porous PVA hydrogel with open pores and channels showed similar EWC and mechanical strength to swine articular cartilage. Such a device in cartilage repair would delay further continued degeneration, and in turn, more invasive surgical treatments.

References:[1] Bodugoz-Senturk, H et al. Biomaterials, 2008, 29(2): 141-9. [2] Bichara DA, et al. *Tissue Eng Part A*. 2011, (3-4):301-9. [3] Ruberti J et al. US Pub. No. 200040092653, 2004 May 13, 2004. [4] Ward I, et al. *An Introduction to Mechanical Properties of Solid Polymers.*2004, (4):64.[5] http://rsbweb.nih.gov/nih-image/.