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# Incorporation of bioactive polyvinylpyrrolidone–iodine within bilayered collagen scaffolds enhances the differentiation and subchondral osteogenesis of mesenchymal stem cells

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ABSTRACT

Polyvinylpyrrolidone–iodine (Povidone-iodine, PVP-I) is widely used as an antiseptic agent for lavation during joint surgery; however, the biological effects of PVP–I on cells from joint tissue are unknown. This study examined the biocompatibility and biological effects of PVP–I on cells from joint tissue, with the aim of optimizing cell-scaffold based joint repair. Cells from joint tissue, including cartilage derived progenitor cells (CPC), subchondral bone derived osteoblast and bone marrow derived mesenchymal stem cells (BM-MSC) were isolated. The concentration-dependent effects of PVP–I on cell proliferation, migration and differentiation were evaluated. Additionally, the efficacy and mechanism of a PVP–I loaded bilayer collagen scaffold for osteochondral defect repair was investigated in a rabbit model. A micromolar concentration of PVP–I was found not to affect cell proliferation, CPC migration or extracellular matrix production. Interestingly, micromolar concentrations of PVP–I promote osteogenic differentiation of BM-MSC, as evidenced by up-regulation of RUNX2 and Osteocalcin gene expression, as well as increased mineralization on the three-dimensional scaffold. PVP–I treatment of collagen scaffolds significantly increased fibronectin binding onto the scaffold surface and collagen type I protein synthesis of cultured BM-MSC. Implantation of PVP–I treated collagen scaffolds into rabbit osteochondral defect significantly enhanced subchondral bone regeneration at 6 weeks post-surgery compared with the scaffold alone (subchondral bone histological score of $8.80 \pm 1.64$ vs. $3.8 \pm 2.19$, $p < 0.05$). The biocompatibility and pro-osteogenic activity of PVP–I on the cells from joint tissue and the enhanced subchondral bone formation in PVP–I treated scaffolds would thus indicate the potential of PVP–I for osteochondral defect repair.
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## 1. Introduction

Osteochondral defects are often observed in traumatic joint injury arising from sports and other rigorous physical activities. Polyvinylpyrrolidone–iodine (PVP–I, betadine), a broad-spectrum biocidal agent, has been widely used for joint lavation in surgery since the 1950s [1–4]. However, the biological effect of PVP–I on joint tissue cells is unclear. Also the effect of PVP–I on the efficacy of the scaffold for osteochondral defect repair has not been investigated.

The biocompatibility of PVP–I has been a controversial issue in previous studies [1–11]. It has been shown that PVP–I inhibits fibroblast growth during wound healing when used as a skin antiseptic [5]. When applied during joint surgery, PVP–I can increase the amount of synovial fluid and cytosis in rheumatoid arthritis [6]. It has also been reported that PVP–I can be damaging to DNA synthesis in chondrocytes [7–10]. Conversely, a recent study by Schmidlin and colleagues indicated that a single short exposure to a 1:10 PVP–I solution enhanced osteogenic differentiation

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in an osteoblast-like cell line MC3T3-E1, albeit with reduced cell proliferation [11]. These conflicting data on the beneficial and detrimental effects of PVP–I warrant further investigation of its biocompatibility and biological impact on cells from joint tissue.

Several types of joint tissue cells are involved in the osteochondral defect repair process. Bone marrow derived mesenchymal stem cells (BM-MSC) are the major intrinsic repair seed cells [12–15]. Other progenitor cells, including cartilage derived progenitor cells (CPC), were also found to be activated upon cartilage injury [16], and osteoblasts from subchondral bone could also provide the appropriate signal for stimulating osteochondal defect repair [17]. Currently, not enough data are available to predict the effect of PVP–I on these joint tissue cells after joint lavation.

Subchondral bone located beneath cartilage tissue plays an important role in osteochondral repair and regeneration. Subchondral bone can attenuate the mechanical stress in joints, and also provide structural support, in addition to performing vital metabolic functions [18]. In large joint osteochondral defects, the defect often fails to heal spontaneously, and the implantation of biomaterial scaffold is often used to facilitate subchondral bone regeneration [17,19–20].

In previous studies, collagen scaffolds have been used for repairing osteochondral defects by facilitating in-growth of surrounding tissues and enhancing regeneration of bone defects through the recruitment of BM-MSC from bone marrow [13–14,21]. However, the tissue structure and function within the central area of large defects following transplantation of collagen scaffolds are still very inferior to those of normal tissue [13,21]. There is, therefore, a dire need to improve the function of scaffold materials in order to achieve desirable outcomes in osteochondral repair and regeneration. And in those cases of joint lavation with PVP–I, it is necessary to evaluate the effects of PVP–I on the efficacy of the scaffold for osteochondral repair.

This study investigated the biocompatibility and biological effects of PVP–I on joint tissue cells, with the aim of optimizing cell scaffolds based osteochondral defect repair. The investigation included: (1) the threshold safe dosages of PVP–I for cartilage and CPC; (2) the effects and mechanisms of action of PVP–I in modulating osteogenesis of BM-MSC and subchondral bone derived osteoblasts in vitro; (3) comparison of the efficacy of bilayered collagen scaffolds with or without PVP–I for osteochondral defect repair in a rabbit model.

## 2. Materials and methods

### 2.1. Scaffold fabrication

#### 2.1.1. Silk scaffold fabrication

To provide the BM-MSC with a proper osteogenic environment in vitro, the three-dimensional (3-D) scaffold was fabricated from natural materials with silk or collagen type I. The porous silk scaffold was fabricated using a salt/water method, as described previously [22,23]. Briefly, raw silk (*Bombyx mori*, Zhejiang Cathaya International Inc., Hangzhou, China) was degummed in a mixture of sodium carbonate (0.08% w/v) and Marseille soap (0.12% w/v) solution at 95 °C for 2 h, and then dissolved in a 9 M lithium bromide solution to a concentration of 20% (w/v) at 60 °C for 4 h. This was followed by dialysis against distilled water for 3 days at 4 °C with a cellulose membrane (MWCO 14,000). The final concentration of the fibroin/water solution was 5.0% (w/v). Salt particles were sieved with 300–400 μm meshes and packed into a plastic syringe. Immediately after a 5.0% (w/v) fibroin aqueous solution was poured into the syringe, the piston was pressed. The silk/salt composite was stored at 4 °C for 24 h, then placed into water to leach out any salt particles. The scaffold was cut with a razor blade to obtain segments 2 mm high.

#### 2.1.2. Collagen scaffold fabrication

The biodegradable collagen sponge used in this study was fabricated from insoluble collagen type I, as reported previously [13,21]. Briefly, collagen type I was isolated and purified from pig Achilles tendon and dissolved in 0.5 M acetic acid (1.0% w/v). The collagen solution was frozen at −80 °C and lyophilized and shaped into a cylinder 4 mm in diameter and 3 mm long for implantation [24].

#### 2.1.3. Field emission scanning electron microscopy and compositional analysis

The dry scaffold was immersed in the solution of 10 μM PVP–I (Sigma, CAS No. 25655-41-8, MW 364.9) for 30 min, then washed in double distilled water three times for 5 min. After overnight drying, scanning was carried out by field emission scanning electron microscopy (FE-SEM) with an energy and angle selective backscattered (EsB) detector. The iodine element within the collagen sponge was analysed by FE-SEM, with the EsB detector for compositional information (FE-SEM, ULTRA55, Carl Zeiss, Germany; equipped with an INCA energy-dispersive X-ray analysis system, Oxford).

### 2.2. Cell isolation and culture

Primary human articular cartilage ($n = 3$, age 65–82 years) chondrocytes and osteoblasts were isolated from total knee joint replacement with ethical approval of the Queensland University of Technology and the Prince Charles Hospital Ethical Committee. The chondrocytes were harvested from the non-lesion area of the joint cartilage surface according to previously described methods [25,26]. The human cartilage-derived colony-forming cells were designated as CPC. The CPC were multiclonally selected and characterized by multipotent differentiation assay and fluorescence-activated cell sorting analysis, according to previous protocols. The colony formation was observed by optical microscopy with crystal violet staining. Osteogenesis was confirmed by alkaline phosphatase (ALP) staining, while the accumulated intracellular lipid droplets were detected by Oil Red O staining [14,27].

For subchondral bone osteoblasts (OB), bone specimens were excised within 5 mm of the subchondral bone plate, as described previously [26]. Primary human bone marrow derived mesenchymal stem cells (hBM-MSC) ($n = 5$, age 27–46 years) were isolated from total knee joint replacement, as described previously [28] with ethical approval of the Institutional Review Board of Zhejiang University. Briefly, BM-MSC were separated and cultured from harvested bone marrow samples ($n = 5$, age = 27–46 years). The colony-forming cells were further cultured at a density of $2 \times 10^4$ cells ml$^{-1}$ in 75 cm$^2$ flasks. Primary cultured cells from the second to fifth passages were used for all subsequent experiments.

### 2.3. Cell behaviour upon treatment with PVP–I

#### 2.3.1. Cell viability assay

Cell viability was assessed using the MTT assay. Briefly, $1 \times 10^5$ cells were seeded in 24-well plates and cultured with Dulbecco's modified Eagle medium (DMEM) in the presence or absence of varyious concentrations of PVP–I (0.1, 1, 10 and 50 μM) at 37 °C for up to 72 h. MTT reagent (Sigma) was added for 4 h and reduction of MTT was assessed at 24 h intervals by a spectrophotometer at a wavelength of 540 nm.

#### 2.3.2. Cell migration assay

Migration of CPC ($n = 3$) was assessed using a Transwell system (Costar 3422) with three technical replicates, as described previously [29]. Briefly, $1 \times 10^5$ cells reconstituted in 100 μl of

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