Osteoarthritis and Cartilage xxx (2014) 1–8

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Arthroscopic airbrush assisted cell implantation for cartilage repair in the knee: a controlled laboratory and human cadaveric study

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ARTICLE INFO

Article history:
Received 13 June 2014
Accepted 5 September 2014

Keywords:
Arthroscopic
Airbrush
Spray
Chondrons
Cartilage repair
Knee

SUMMARY

Objective: The objective of this study was to investigate the feasibility of arthroscopic airbrush assisted cartilage repair.
Methods: An airbrush device (Baxter) was used to spray both human expanded osteoarthritic chondrocytes and choncrocytes with their pericellular matrix (chondrons) at 1×10^6 cells/ml fibrin glue (Tissucol, Baxter) *in vitro*. Depth-dependent cell viability was assessed for both methods with confocal microscopy. Constructs were cultured for 21 days to assess matrix production. A controlled human cadaveric study ($n = 8$) was performed to test the feasibility of the procedure in which defects were filled with either arthroscopic airbrushing or needle extrusion. All knees were subjected to 60 min of continuous passive motion and scored on outline attachment and defect filling.
Results: Spraying both chondrocytes and chondrons in fibrin glue resulted in a homogenous cell distribution throughout the scaffold. No difference in viability or matrix production between application methods was found nor between chondrons and chondrocytes. The cadaveric study revealed that airbrushing was highly feasible, and that defect filling through needle extrusion was more difficult to perform based on fibrin glue adhesion and gravity-induced seepage. Defect outline and coverage scores were consistently higher for extrusion, albeit not statistically significant.
Conclusion: Both chondrons and chondrocytes can be evenly distributed in a sprayed fibrin glue scaffold without affecting viability while supporting matrix production. The airbrush technology is feasible, easier to perform than needle extrusion and allows for reproducible arthroscopic filling of cartilage defects.

Introduction

Articular cartilage defects are a known cause of disability in young and active patients and treatment of these challenging injuries is becoming standard of care in orthopedic practice. Current algorithms include microfracture for smaller defects and autologous chondrocyte implantation (ACI) or osteochondral autograft transfer system (OATS) for larger defects (>2.5 cm^2)[1,2]. ACI is capable of stimulating hyaline-like tissue regeneration with good clinical results up to 20 years[3]. Since ACI was first introduced in the early nineties, different generations have been developed to improve structural and patient outcomes as well as surgical handling[4,5]. Today, third generation ACI, which uses a collagen or hyaluronic acid scaffold, is one of the most widely used cell-based therapies in sports medicine. Although an arthroscopic approach has been described, it is only performed in 'expert hands'[2,6]. Again, these treatments are successful in the mid to long-term follow-up but require two surgical procedures and are expensive[7]. What's more, the focus in regenerative medicine has primarily been on optimizing cellular products, and not on improving surgical techniques, creating a gap in translation of new technologies into clinical practice. Consequently, surgeons mainly use a

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http://dx.doi.org/10.1016/j.joca.2014.09.016
1063-4584/

mini-arthrotomy when performing ACI because arthroscopic manipulation of cell-laden (hydrogel) scaffolds and their subsequent fixation remains a challenge[8,9]. In the last few years, focus has gone from a two-stage to a single-stage procedure as this is of less burden on the patient and could be more cost-effective in the long term. For example, a recent study showed positive early clinical and radiological outcome after carbon dioxide insufflated arthroscopic microdrilling and collagen/fibrin gel application for large (2–8 cm^2) defects[10]. In addition, single-stage procedures using stem cells are increasingly being introduced in clinical trials[11,12]. Recently, we have completed the inclusion of a phase I/II clinical trial (IMPACT trial, clinicaltrials.gov: NCT02037204, http://www.youtube.com/watch?v=S3rIBj A03AA) in which chondrons and mesenchymal stromal cells (MSCs) are mixed in a fibrin glue scaffold and implanted within one surgical procedure. Gel-based cellular therapies often require an open surgical procedure as the slope of the femoral condyles create difficulty in keeping the scaffold in place whilst gelating[13]. As it is known that arthroscopy is less invasive and has a faster recovery time and shorter in-hospital stay compared to an open procedure[14], introducing an easy to handle gel application system would be highly valuable. Our proposed airbrush technology platform may allow for an arthroscopic delivery of gel which immediately gelates and adheres to the subchondral bone under any slope in any joint. Spray-applied cell therapy has already found its way in other fields and for example, was shown capable of healing venous leg ulcers in a recent randomized controlled trial[15]. The purpose of this study was to investigate the feasibility of spraying cell-based fibrin glue scaffold in an arthroscopic cadaver model.

Material and methods

Study outline

To evaluate the hypothesis that airbrushing does not affect cell survival we conducted a laboratory study in which we performed a viability assay to compare cell death after airbrushing and needle injection ($n = 4$). Using a glycosaminoglycan (GAG) assay, we wanted to test whether the airbrush system would affect cartilage regeneration *in vitro*. Once the *in vitro* study was finished, we started a controlled laboratory study to investigate the surgical feasibility of the arthroscopic airbrush approach in comparison to needle extrusion.

Donors

Cartilage was obtained from redundant material from five patients undergoing total knee arthroplasty. The anonymous use and collection of this material was performed according to the Medical Ethical regulations of the University Medical Center Utrecht and the guideline 'good use of redundant tissue for research' of the Dutch Federation of Medical Research Societies.

Cell isolation

Chondrocytes with their pericellular matrix (chondrons) were acquired using a two hour rapid digestion protocol adapted from Bekkers *et al.*[16] Briefly, the cartilage was cut into small pieces, rinsed in phosphate buffered saline (PBS) (Life technologies, UK) and digested for two hours in 1% Collagenase type II (Worthington, Lakewood New Jersey) at 37°C in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Bleiswijk, the Netherlands) with 1% penicillin/streptomycin (100 U/mL/100 μg/mL; Invitrogen, Life Technologies). After digestion, the suspension was filtered through a 100 μm cell strainer (BD Biosciences, San Diego, CA, USA), the chondrons were washed once in PBS, spun by centrifugation at 300 *g* in ten minutes and resuspended in 100 μL PBS. For comparison, chondrocytes were isolated overnight using 0.15% Collagenase type II (Worthington, Lakewood New Jersey) using the same medium and washing procedure.

Preparation of the cell containing fibrin glue

For the application of cells/chondrons, a commercially available, clinical grade fibrin glue (Tissucol Duo 500, Baxter) kit was used which consists of a syringe containing a solution comprising fibrinogen, fibronectin, plasminogen and factor XIII, and one containing thrombin. After mixture, the components gelate immediately upon injection and gelation is complete after approximately 30 s. After isolation (Passage 0) cells were resuspended in the fibrinogen component at an overall concentration of 2×10^6 cells/ml fibrin glue and placed next to the thrombin syringe.

Airbrush and controls

For the airbrush application, the two-component system was connected to a clinically approved airflow device (DuploSpray MIS, Baxter) which regulates the outflow of compressed air (1 bar) through sterile filters. The cell-laden fibrin glue was sprayed at a distance of approximately 2 cm in 24-well plates at a doses of 500 μl per well. As a control, 500 μl the fibrin glue was extruded through a needle into the well. Per donor, three samples were used for each condition. After the cell-laden fibrin constructs were made, 1 ml of culture medium was added. Culture medium consisted of DMEM supplemented with 2% insulin-transferrine-selenium (ITS)-X (Invitrogen), 2% l-ascorbic acid-2-phospate (AsAp; Sigma–Aldrich), 2% human serum albumin (HSA; Sanquin Blood Supply Foundation Amsterdam, the Netherlands), 1% penicillin/streptomycin (100 U/mL/100 μg/mL) and 1 ng/ml TGF-β2 (R&D Systems, Minneapolis, Minnesota).

Assessment of cell viability

To evaluate the effect of spraying on cells, a viability assay using a live/dead staining kit for mammalian cells (Molecular Probes, Eugene, OR, USA) was performed according to the manufacturer's instruction after application. The fibrin hydrogel constructs were washed 3 times with DMEM and transferred to a microscopy slide to be visualized with a fluorescence microscope. Live (green) and dead cells (red) were counted by two observers (TdW and JB) independently. If there was a discrepancy in scoring, a consensus was reached. To test the hypothesis that cells impacted on the solid surface would be less viable (depth dependent viability), both chondrons and chondrocytes ($n = 4$) were sprayed (two samples per condition) into a six well chamber slide (Lab-Tek, Nalge Nunc International), subjected to a viability assay and observed using laser scanning confocal microscopy with a construct height (Z) of up to 2600 μm. Leica TCS SP5 inverted confocal microscope equipped with a HCX PL APO CS 63×/1.20-0.60 OIL objective (Leica Microsystems, The Netherlands). During visualization, dyes were excited simultaneously with a 488-nm argon laser and detected using two separate photomultipliers set to appropriate bandwidths. Slides were bundled and divided in quartiles. The cell distribution and viability was compared between the lower and upper quartile.

Cell culture and glycosaminoglycan analysis

Chondron laden fibrin glue was sprayed ($n = 4$) or extruded ($n = 4$) and cultured for 21 days (two samples per donor). The culture medium was changed twice a week. After the 3-week

Please cite this article in press as: de Windt TS, et al., Arthroscopic airbrush assisted cell implantation for cartilage repair in the knee: a controlled laboratory and human cadaveric study, Osteoarthritis and Cartilage (2014), http://dx.doi.org/10.1016/j.joca.2014.09.016