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

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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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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

culture, samples were digested overnight at 60°C in a papain digestion buffer (250 μg/ml papain (Sigma–Aldrich), 0.2 M NaH_2PO_4, 0.1 M EDTA, 0.01 M cysteine) prior to quantification of the GAG content with a dimethylmethylene blue (DMMB) assay with an absorption ratio of 540 nm–595 and chondroitin-6-sulphate (Sigma–Aldrich) as a standard. DNA content in the papain digests was determined using a Picogreen DNA assay (Invitrogen) according to the manufacturer's instruction.

Cadaveric study

To test the surgical feasibility of the arthroscopic airbrush system, a controlled human cadaveric study was performed in eight different knee joints of six male and two female donors (age range 56–88) in accordance with the guidelines of the local ethical committee. The specimens were derived from bodies who entered the Department of Anatomy through a donation program. From these persons, written informed consent was obtained during their lifetime that allowed use of their bodies for educational and research purposes. Each knee was tested on (visual) malalignment, stability and range of motion (full extension and at least 80° flexion). A mini-arthrotomy of approximately 5 cm was performed using a medial parapatellar approach to obtain a view of the medial femoral condyle while preventing leakage of synovial fluid. A template was used with a diameter of 1.6 cm (square surface 2.0 cm^2) to create standard sized full-thickness cartilage defects. Using a sharp spoon, the defect was debrided and the calcified layer removed. The knee was closed in layers prior to performing a carbon dioxide (CO_2) insufflated arthroscopy (Smith & Nephew) with a standard anterolateral approach. The two-component holder of the cell laden (2×10^6 cells/ml) fibrin glue was connected to the DuploSpray system which was originally designed to spray a fibrin sealant as a hemostatic agent for laparoscopic surgery[17,18]. For the cadaveric study, both chondrons ($n = 3$) and chondrocytes ($n = 5$) were used. The fibrin glue was stained with Fast Green FCF (Merck Millipore, Darmstadt, Germany) and randomly applied to the cartilage defect, using either the airbrush system ($n = 4$), or a standard Duplotip syringe (Baxter) ($n = 4$) utilizing CO_2 insufflation. Afterwards, each leg was subjected to a continuous passive motion (CPM) protocol[19,20]. Briefly, the legs were firmly strapped onto a CPM machine (Medical sot B.V., Losser, the Netherlands) and subjected to 60 cycles of full extension to 80 degree flexion in one hour. After the CPM protocol, the knees were reopened and photographed for macroscopic analysis. Next, the fibrin glue was thoroughly removed from the defect site, the knee closed in layers and the same cartilage defect was used for arthroscopic application of the alternative treatment followed by the CPM and evaluation protocol. The knees were scored by four independent observers (blinded for the application method) using a modified macroscopic scoring system adapted from previous work (Table I)[19,20]. To assess the reliability of the macroscopic scoring system, inter-observer agreement was determined as well as test–retest analysis (intra-observer agreement) with a 2-month interval.

Cryomicrotome sectioning

To assess the defect fill and the local dispersion of the cell laden fibrin glue after the arthroscopic airbrush procedure two random specimens were used for cryomicrotome sectioning. In short, the specimens were frozen overnight, followed by embedding *en block* in 1% carboxymethylcellulose (CMC) at −25°C for 24 h. Cryosections were obtained using a cryomicrotome (CM3600 XP; Leica Biosystems, Nussloch, Germany) with a 50 μm interval. The sections were made in a in a transverse plane for one specimen and a frontal plane for the other. Each section was photographed (Leica DFC 450 C, Leica the Netherlands) and photographs were bundled for evaluation.

Table I
Modified macroscopic scoring system

Outline attachment*	Area coverage†
100% (5)	Unchanged (100%) (5)
75–100% (4)	75–100% (4)
50–75% (3)	50–75% (3)
25–50% (2)	25–50% (2)
0–25% (1)	<25% (1)
0% (0)	0% (0)

* % of full circumference that has contact with the surrounding cartilage rim.
† % of total cartilage defect that is covered by fibrin glue.

Statistical analysis

Interdependent Samples *t*-tests were used to compare the viability and matrix production between the extrusion and airbrush method. To determine both the inter-rater and the intra-rater (test–retest) agreement for the macroscopic scoring of the extrusion and airbrush application, the mean (Fleiss–Cohen) weighted K was used[21–23]. A K value of <0.20 was considered to indicate slight agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 substantial agreement and 0.81–0.99 almost perfect agreement[24]. For the inter-rater agreement, the outline and coverage scores were compared between all observers and mean K values were determined along with the standard deviation. Similarly, test–retest scores were determined for each individual observer and K values were averaged. Chi-square analysis was conducted to compare the macroscopic scores between both application methods per individual rater. For the statistical analysis, Excel 2007 (Microsoft) and SPSS version 20 (IBM SPSS Inc. Chicago, Illinois) were used.

Results

Assessment of cell viability

Airbrush applied scaffolds could be centralized and contained within the wells of the 24 well plate without cells leaving the fibrin glue as confirmed by microscopy. The mean cell viability of chondrons for the airbrush group was 76.7% (confidence interval (CI) [66.6, 86.7]) and 73.8% (CI [62.4, 85.3]) for the extrusion group ($P = 0.69$) (Fig. 1) Confocal microscopy showed an even distribution of live and dead cells through the sprayed fibrin construct and no difference in viability between cells impacting on the solid surface (lower quartile viability 88.4% (CI [78.1, 92.7])) and the cells in the superficial layer of the fibrin construct (upper quartile viability 85.1% (CI [76.7, 93.6])) $P = 0.87$ (Fig. 2). When chondrons were replaced by chondrocytes, confocal microscopy demonstrated comparable overall cell viability results with an average cell viability for airbrush of 80.8% (CI [69.9, 91.7]) and 78.8% (CI [67.9, 89.8]) for extrusion $P = 0.83$. When comparing the viability after spraying between chondrons (76.7% (CI) [66.6, 86.7]) and chondrocytes (80.8% (CI [69.9, 91.7])) no statistically significant difference was found $P = 0.66$.

Cell culture and glycosaminoglycan analysis

The airbrush system did not affect the capability of cultured chondrocytes to produce GAGs as the average produced GAG per DNA was 1.19 (CI [0.33, 2.04]) after spraying and 0.59 (CI [0.11, 1.08])

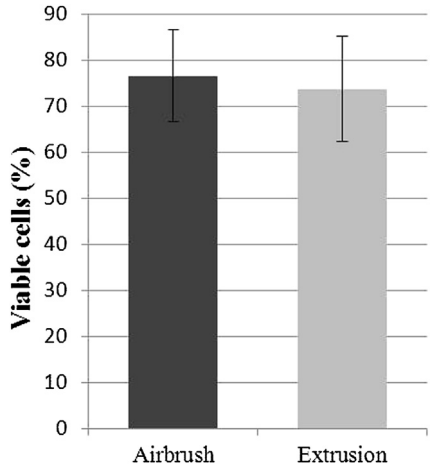

Fig. 1. Cell viability (average % and 95% confidence interval) for the airbrush and extrusion application ($n = 4$).

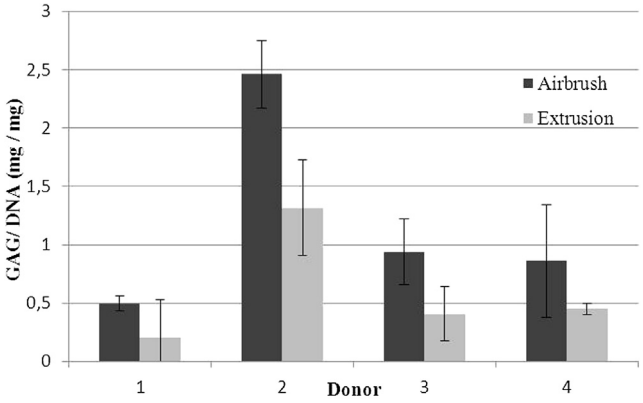

Fig. 3. Glycosaminoglycan (GAG) per DNA (average and 95% confidence interval) for the 21-day fibrin glue cultures ($n = 4$). Comparison between airbrush and extrusion.

0.49 after extrusion. The difference in mean GAG per DNA was not statistically significant ($P = 0.28$) (Fig. 3).

Cadaveric study

Arthroscopic airbrushing was found to be a feasible surgical procedure which was easy to perform and allowed for a local delivery with containment in the defect. Arthroscopic needle extrusion was more challenging to perform as gravity induced seepage and unintentional adhesion of the construct to the needle disturbed surgical ease. No difference in gross appearance or gelation time was observed when chondrons or chondrocytes were used. For the four observers, the average outline and coverage score for the airbrush method was 4.25 (CI [3.84, 4.66]) and 4.31 (CI [3.98, 4.65]), respectively. The average outline and coverage score for extrusion was 4.13 (CI [3.65, 4.60]) and 3.84 (CI [3.09, 4.60]), respectively [Fig. 4(A) and (B)]. Figure 5 provides pictures of the best and worst results in terms of defect fill for both application methods. For needle extrusion the worst case demonstrates a half empty defect which was a result of part of the construct adhering to the needle. Although the macroscopic scores were slightly higher for airbrushing, especially for the coverage scores, chi-square analysis showed that differences between both application methods were not significant (P range 0.20–1.00). The inter-rater agreement was good (mean $K > 0.70$) for the macroscopic outline score and moderate to excellent (mean K range 0.54–0.88) for the coverage score (Table II). Similarly, the intra-rater (test–retest) reliability was good for the outline scores (mean $K > 0.70$) and moderate to good

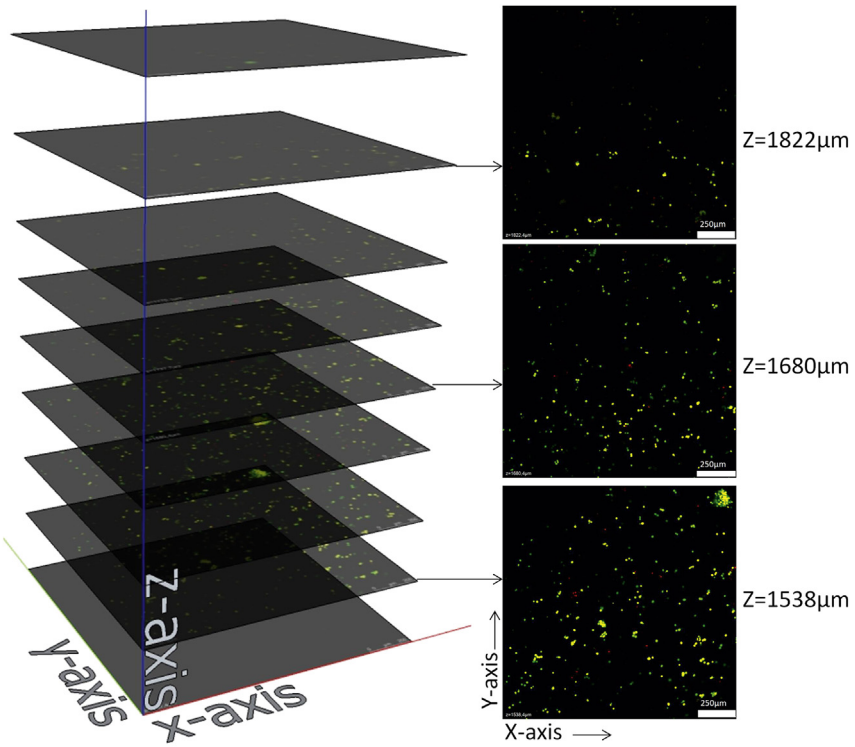

Fig. 2. Confocal microscopy after the airbrush application of chondron laden fibrin glue ($n = 4$). A homogenous distribution was found with an equal survival in the lower, central and superficial layer. Green and red staining indicated live and dead cells, respectively.

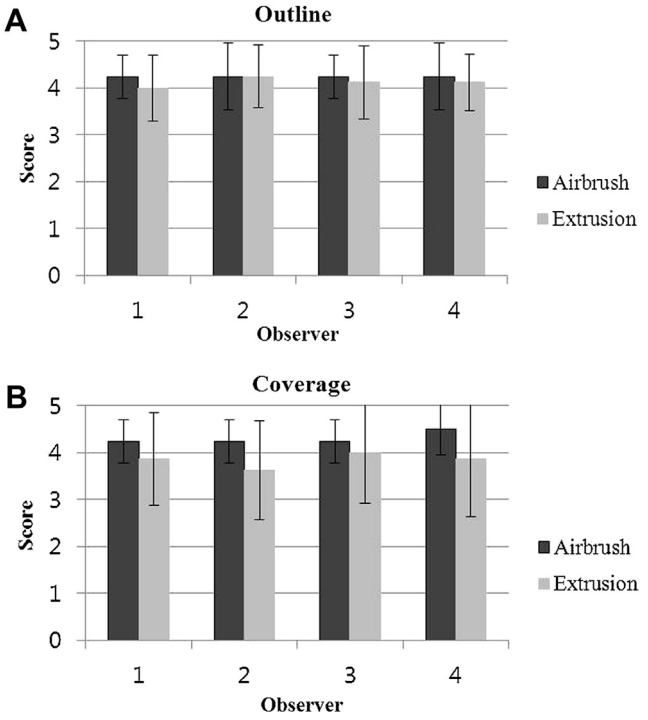

Fig. 4. A, Modified macroscopic outline score (average and 95% confidence interval) for the different observers (n = 8 specimens). B, Modified macroscopic coverage score (average and 95% confidence interval) for the different observers (n = 8 specimens).

(mean K range 0.55–0.76) for the coverage scores (Table II). The cryomicrotome analysis showed a complete fill of the defect without migration of the graft (Supplemental Movie 1 and 2).

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.joca.2014.09.016.

Discussion

The most important finding of the present study is that arthroscopic cell therapy using an airbrush system does not affect cellular viability and has good surgical feasibility. Compared to direct needle extrusion, airbrushing was found to have superior surgical handling, good defect fill and similar outline and coverage scores. Needle extrusion was surgically unpractical due to gravity induced seepage and unintentional adhesion to the needle [Fig. 5(D)].

The rapid advancements in regenerative medicine have not yet led to a cartilage repair method with easy arthroscopic handling[5]. Spray-applied cell therapy has gained more attention in recent years, as it is easy to use, allows for fast gelation, good adherence and local cellular regeneration[25]. In their randomized controlled trial Kirsner *et al.*[15] demonstrated an added value of using cells (keratinocytes and fibroblasts) in a spray applied fibrin glue for the treatment of chronic venous leg ulcers. Two separate studies showed that a cell-laden fibrin spray can safely and effectively be delivered to (burn) wounds[26,27]. Using the same airbrush system as in the present study, adipose derived MSCs were also demonstrated to be capable of forming endothelial tubules *in vitro* realizing an easy to handle approach for vascular and dermal regeneration[25]. Surprisingly, none of the above-mentioned studies provided viability data, which in terms of scientific validation, is of great importance for such cellular therapies. Thus to the best of our knowledge, the present study is the first to demonstrate that the viability of cells is not affected by spraying in a fibrin carrier. Fibrin glue is a scaffold known for its regenerative capacity for cartilage, bone and dermal regeneration[28–30]. Despite the fact that it is widely used, and has been proven to be safe and effective *in vivo*, it may be that hydrogels which have a slower degradation process could further improve implant stability[13,31,32]. For example, photopolymerizable PEG hydrogels have shown up to 10-times stronger adhesion to articular cartilage in comparison to fibrin glue[33,34]. A hydrogel suitable for a (arthroscopic) spray application needs both immediate gelation upon the aerosol mixing state and good adherence. It thus may well be that biomechanically improved hydrogels with self-attaching and cell-attracting properties are equally suitable for an airbrush application[35]. Perhaps more importantly, the proposed airbrush system can serve as a platform for further development of an easy to handle approach for larger cartilage defects using an optimal hydrogel. Other arthroscopic (single-stage) gel-based procedures may also benefit from an airbrush application as the slope of the femoral condyle and gravity create difficulty when inserting hydrogels. For instance, the surgical handling of the collagen/fibrin gel covered microfracture as recently introduced by Shetty *et al.*[10] could be improved by using an airbrush system. However, this microfracture technique may be less suitable for larger defects, as they may respond better to osteochondral autologous transfer or cell therapy[36,37].

There are some critical remarks to be made regarding the present study. Although we did not find an effect of airbrushing on viability and matrix production, we cannot rule out other (subtle) cellular responses airbrushing has on cells. For example, it is known that increased (hydrostatic) pressure can influence matrix gene expression of expanded chondrocytes[38–40]. However, it should be mentioned that these studies used pressures ranging from 5 to 100 Bar where we have used an air pressure of only 1 bar. Indeed, it could be that viability is affected only at higher pressures, such as previously shown for bioprinting, or that fibrin glue provides protection upon impaction[41]. Moreover, the finding that chondrocytes show reversible pressure-induced cytoskeletal remodeling could suggest there may only be a temporary response to spraying which we have not observed[42]. It may be valuable to investigate the effect spraying has on cells on a more detailed level to determine optimal spray conditions.

In the present study, chondrons were used for the proposed cellular therapy. Chondrons are chondrocytes with their pericellular matrix which have been shown to have a better capability of cell-induced cartilage production compared to chondrocytes both *in vitro* and in a small animal model[16,43–45]. The fact that chondrons were found to be mechanically stiffer than chondrocytes could mean that chondrons are better suitable to undergo increased pressure upon spraying[46]. Nevertheless, we did not encounter a statistically significant difference in confocal microscopy evaluated depth-dependent survival between chondrons and chondrocytes. Moreover, as a variety of cells were found capable of inducing tissue regeneration after a spray application, it is likely that different cell types can be used for spraying[15,25–28,47,48].

As we have performed a cadaver study, we do not know the effects spraying fibrin glue will have on integration with the native tissue. Securing integration of cellular transplants is a known challenge that needs to be addressed[33]. Still, as we have found both good defect fill and cellular distribution, we do not anticipate integration would differ between sprayed and non-sprayed transplants.

In this study we have used a CPM model that is similar to our non-weight bearing protocol immediately after surgery. It could be however, that the (muscle) strain on the transplant is higher *in vivo*. Nonetheless, cartilage tissue regeneration using fibrin glue incorporated cell transplantation has been shown after immediate weight bearing in animal models[16,49] and standard (delayed) weight bearing protocols in early clinical trials[13,50].

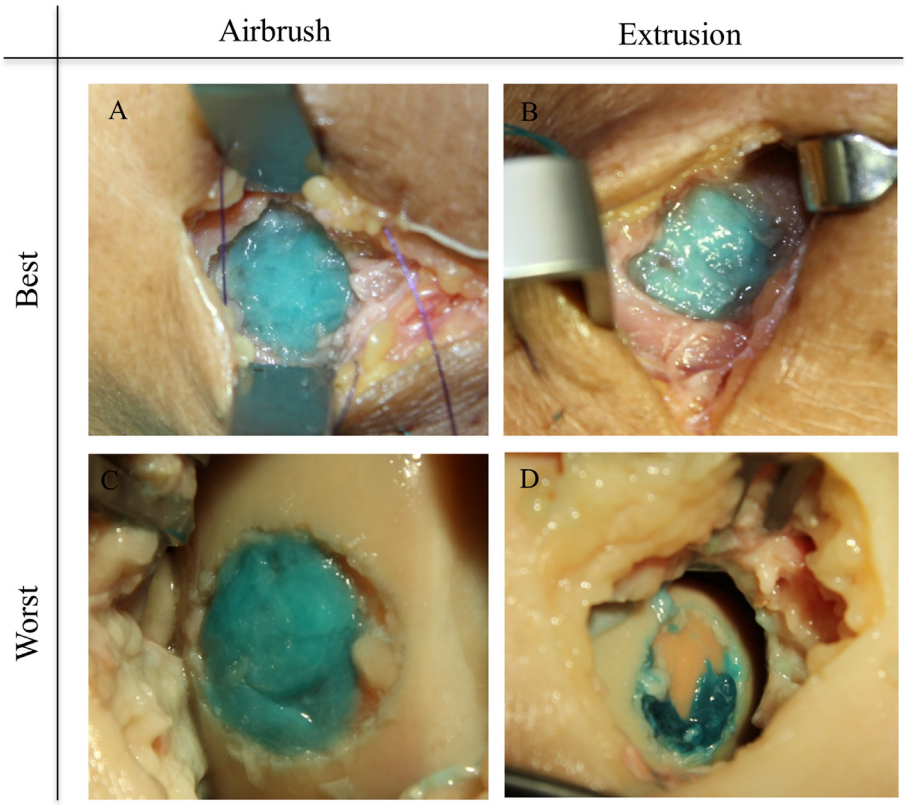

Fig. 5. Pictures showing a full-thickness cartilage defect on the medial femur condyle in a human cadaveric knee with the best defect coverage and outline results after both airbrushing (A) and needle extrusion (B). The worst results for both methods are shown in C and D.

A possible limitation of using the airbrush system is that it requires a CO_2 insufflated arthroscopy. This is especially so when a water-assisted concomitant procedure such as shaving is required. Our pilot study has taught us that it is feasible to perform a water-insufflated arthroscopy prior to utilizing CO_2 insufflation, though the defect needs to be blown dry before spraying. CO_2 insufflation was recently shown to be a safe technique capable of advancing surgical comfort compared to traditional fluid arthroscopy without a higher risk of gas embolism[51]. The authors suggested that the use of a pneumatic tourniquet avoids a potential systemic diffusion of gas. The additional safety concerns regarding spray-applied cell therapy in the knee are minimal. According to the Food and Drug Administration (FDA), the occurrence of air embolisms (in nine patients) was associated with laparoscopic application of spray-applied sealants with higher than recommended pressures and too close proximity[52]. From these patients, one received the same sealant as we have used in this study, and no clear relationship with the procedure was found. What's more, the cellular spray application on a minimally vascularized bony surface may better resemble topical spray-applied cell therapy, which for venous ulcers and burn wounds, has been shown to be safe without the occurrence of embolisms[15,26,27]. Taken the above into account and considering a local use in accordance with current guidelines (pressure 1 bar, 2 cm distance) and the (standard) use of a tourniquet, the risk of using a cellular spray system is considered particularly low. Thus the prospect of providing a surgeon with an extra tool during standard as well as dry arthroscopic procedures further stimulates the development of an airbrush platform for hydrogel-based arthroscopic cartilage repair prior to (animal) testing and clinical introduction, especially since using an inexpensive commercially available spray-system will not have a negative effect on cost-utilities. Here it is important to stress that this is an

Table II
Inter-rater and intra-rater agreement for the macroscopic outline and coverage score

Score	Inter-rater reliability				Intra-rater (test–retest) reliability			
	Application	*n*	*K*	CI	Application	*n*	*K*	CI
Outline	Extrusion	8	0.79	0.72–0.86	Extrusion	8	0.72	0.58–0.85
Outline	Airbrush	8	0.71	0.58–083	Airbrush	8	0.70	0.55–0.86
Outline	Overall	16	0.73	0.67–0.80	Overall	16	0.71	0.63–0.78
Coverage	Extrusion	8	0.88	0.84–0.91	Extrusion	8	0.76	0.61–0.91
Coverage	Airbrush	8	0.54	0.35–0.74	Airbrush	8	0.55	0.45–0.65
Coverage	Overall	16	0.82	0.76–0.87	Overall	16	0.66	0.53–0.79

Inter-rater reliability was calculated as the mean agreement between the different observers and Intra-rater reliability as the mean agreement within each observer with an interval of 2 months.
n, number of specimens scored.
K, Kappa statistic.
CI, 95% confidence interval.

investigator-driven study that used a clinically available system to allow fast clinical translation.

Although there were subtle differences in observer blinded outline and outcome scores in favor of airbrushing, the cadaver study may have been underpowered to show a statistical difference. This subtle difference found, was largely attributable to partial delamination of the graft which was a result of adhesion to the needle upon extrusion. [Fig. 5(D)] Indeed, although it could not be quantified, our study showed that both in experienced and non-experienced hands, airbrush assisted cell therapy was easier to perform with a higher success rate in terms of defect coverage.

Conclusion

This is the first study to show that both chondrons and chondrocytes can be evenly distributed in a sprayed fibrin glue scaffold without affecting cellular viability while supporting matrix production. This stimulates further development of the airbrush system for fast clinical translation and creates a platform for future analysis using mechanically stronger hydrogels for articular cartilage repair.

Disclosures

None to this topic.

Author contributions

Conception and design: de Windt, Vonk, Buskermolen, Visser, Bleys, Saris.

Collection and assembly of data: de Windt, Vonk, Buskermolen, Visser.

Analysis and interpretation of data: de Windt, Vonk, Buskermolen, Visser, Karperien, Dhert, Saris.

Drafting article: de Windt, Vonk, Buskermolen, Visser, Saris.

Revising for important intellectual content: de Windt, Vonk, Visser, Karperien, Bleys, Dhert.

Final approval: de Windt, Vonk, Buskermolen, Visser, Karperien, Bleys, Dhert, Saris.

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Competing interest statement

None of the authors have declared a conflict of interest.

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