Arthroscopic Techniques for the Fixation of a Three-Dimensional Scaffold for Autologous Chondrocyte Transplantation: Structural Properties in an In Vitro Model

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Purpose: The aim of the present study was to evaluate the structural properties of matrix-associated autologous chondrocyte implantation with multiple fixation techniques implanted in fresh porcine knees after they had undergone load to failure. **Methods:** We evaluated the ultimate failure load, yield load, and stiffness of 3 different techniques for the fixation of a 2-mm thick polymer fleece: (1) fixation with biodegradable polylevolactide pins, (2) a transosseous anchoring technique, and (3) conventional suture fixation. Techniques 1 (pin) and 2 (transosseous anchoring) can be used arthroscopically. **Results:** Maximum load and yield load were significantly higher in the group 1 (pin fixation) and group 2 (transosseous anchoring) compared to group 3 (conventional suture). Stiffness was significantly higher in group 1 than in groups 2 or 3. **Conclusions:** Our biomechanical data show that two fixation techniques (pin fixation and transosseous anchoring) have a higher ultimate load, yield load, and stiffness than the conventional suture technique at time point zero. However, these data can be interpreted only with the Bioceed-C matrix (BioTissue Technologies GmbH, Freiburg, Germany). **Clinical Relevance:** Our biomechanical data show outstanding fixation strength with arthroscopic techniques that use Bioceed-C matrix scaffolds during autologous chondrocyte transplantation. Thus, arthroscopic fixation done with this biomaterial should benefit patients, which, in turn, should lead to further research on these arthroscopic techniques and this biomaterial. **Key Words:** Arthroscopy—Biomechanics—Cartilage—Chondral defect—Matrix—Maximal load.

In 1994, Brittberg et al.[1] introduced the autologous chondrocyte implantation (ACI) as a method for the treatment of chondral defects. It seems to be a disadvantage that ACI is an open technique for cartilage repair while other techniques, such as microfracture, can be performed arthroscopically.

From other surgical procedures, it is known that arthroscopic techniques are associated with less postoperative morbidity than open procedures. Adhesions, scar formation, and postoperative pain can be reduced by using an arthroscopic approach.[2] A multicenter study investigating the clinical outcomes after ACI has shown that more than 26% of the procedure-related complications of ACI can be contributed to arthrotomy.[3]

To overcome these disadvantages, arthroscopic techniques for ACI were developed.[2,4] For these arthroscopic techniques, the development of biodegradable matrices that can be seeded with chondrocytes instead of the periosteum was essential. The introduction of matrix-associated ACI (M-ACI) minimizes the donor site morbidity associated with harvesting of the periosteum; it prevents dedifferentiation of the cells during the culturing process and hypertrophy of the periosteum.[5-9]

Different scaffolds are available for clinical use, but there are only a few technical reports about arthroscopic M-ACI in the literature.[2,4,7-9] A problem of all

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these techniques may be fixation of the matrix within the defect, because it is impossible to suture the matrix to the surrounding cartilage via an arthroscopic approach. Erggelet et al.[2] introduced a technically demanding transosseous anchoring technique with the use of a bioabsorbable thread. An alternative could be the use of bioabsorbable pins to fix the matrices to the subchondral bone.[10] These pins have been developed for the refixation of osteochondral segments. As far as we know, biomechanical data about the primary stability of both techniques has not been published.

The aim of the present study was to evaluate the structural properties of M-ACI during arthroscopic fixation using a transosseous suturing technique[2] and a technique using biodegradable pin devices, and to compare the results to conventional suture techniques.

Our hypothesis was that the yield load, maximal load, and stiffness of both arthroscopic fixation techniques at time point zero are superior to these of conventional suture techniques.

METHODS

In this study, a 2-mm thick biodegradable polymer fleece was used as scaffold (Bioceed-C; BioTissue Technologies GmbH, Freiburg, Germany). A 3 cm × 2 cm standard size was used. In this scaffold, chondrocytes can be seeded after suspending the cells in a fibrin gel. However, because the scaffold used mediates the biomechanical properties of such grafts,[11] the scaffold was tested without cells.

Fresh porcine knees were used for testing the stability of the fixation of the scaffold to the bone. The mean age of the animals was 25 ± 2 weeks. The material was obtained from a local butcher, fresh frozen at −20°, and thawed for 12 hours at room temperature before testing. The muscles and soft tissues were removed, leaving the femur intact. The femurs were randomly assigned to 3 different study groups (Fig 1): in group 1, we used fixation with biodegradable pins (SmartNail; ConMed Linvatec, Largo, FL); in group 2, we used the transosseous anchoring technique as described by Erggelet et al.[5]; and in group 3, the conventional suture technique was used, with 6-0 polydiaxanone (PDS) sutures.

A power analysis showed that a group size of 8 would be enough to detect a statistically significant difference. For the fixation of the scaffold, a rectangular chondral defect was created on the medial femoral condyle, using a scalpel and a spoon. Then, one of the 2-cm sides of the matrix was fixed either by suture to the healthy cartilage or by one of the osseous fixation techniques. For the suture technique, four 6-0 PDS sutures were used.

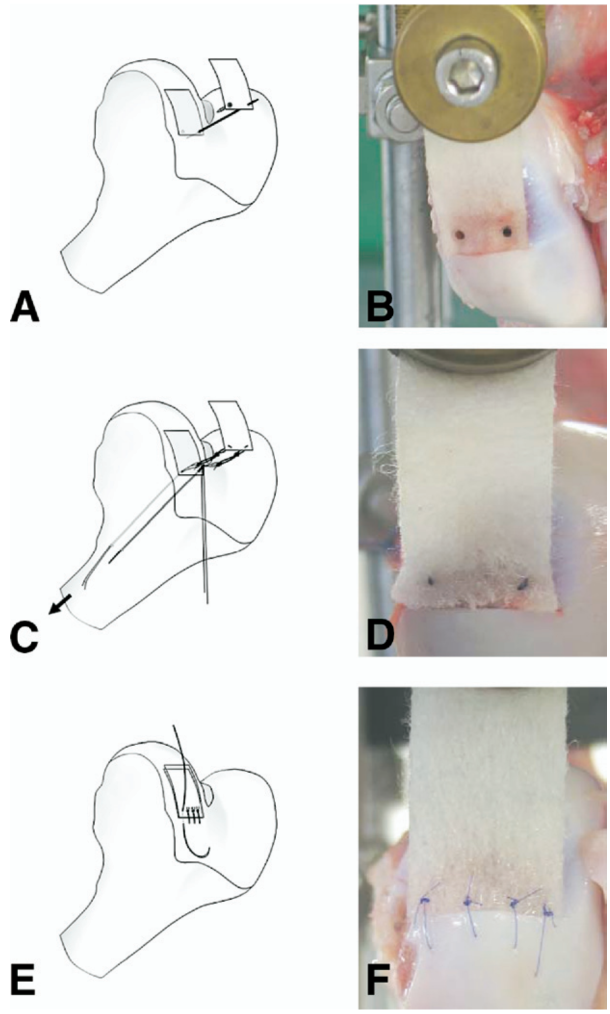

Figure 1. Schematic drawing and pictures of the fixation techniques tested. Pin fixation (A, schematic drawing; B, picture), transosseous suture fixation technique (C, schematic drawing; D, picture), and conventional suture technique (E, schematic drawing; F, picture).

Technique for the Fixation With Biodegradable Pins

The technique for the fixation of the scaffold has been described elsewhere. [10] For the fixation of the matrix to the porcine medial femoral condyle, the matrix was held in place with a test probe. Then a specific drill guide (ConMed Linvatec) was placed on the scaffold in a perpendicular angle. Care was taken not to change the position of the drill guide during the next two steps. The subchondral bone was drilled with a 1.2-mm K-wire. The drill hole had a minimum length of 16 mm. Then the biodegradable pin (16 mm in length) was placed in the drill guide, which can also be used as an insertion instrument for the SmartNail. The pin was carefully tapped into the subchondral bone (Fig 1A and B). Two pins were inserted at each edge of the scaffold. The distance between the pins was measured with a digital calliper.

Transosseous Fixation Technique

The transosseous fixation technique was described by Erggelet et al.[2] For the transosseous fixation techniques, two corners of the artificial defect were drilled with a guidewire. Using a resorbable thread (2-0 *United States Pharmacopeia* [USP]), the scaffold was armed on the two corners. One 3-fold knot, tied approximately 1 cm from the edge, secured the sling. An additional knot, tied approximately 2 cm away, moored the sling, which served as a pulley.[2] The pulley slings were pulled through the femoral bone by the guide wire. Firm action on the pulley guides the knots into the drill holes (Fig 1C and D). The distance between the sutures was measured with a digital calliper.

Conventional Suture Technique

The suture fixation technique was described by Brittberg et al.[1] for the fixation of the periosteum in the original ACI. For this study, the 2-cm edge of the scaffold was sutured with PDS (6-0 USP) to the uninjured cartilage. Four sutures were used for each matrix (Fig 1E and F). The distance between the sutures was measured with a digital calliper.

Biomechanical Testing

During testing, the specimens were moistened with saline solution buffer. All tests were performed at room temperature. Tensile testing was performed using a custom made apparatus mounted in a uniaxial testing frame (Lloyd Instruments, Fareham, England; Fig 2). The scaffold was friction-locked in a custom made fixation clamp. All loads were applied tangential to the surface of the subchondral bone to imitate shear forces.

A preload of 5 N was first applied to the specimens. Then the scaffold–femur constructs were tested to failure. During loading to failure, load and elongation were recorded continuously using a personal computer. The resulting load elongation curve was documented, as well as the ultimate failure load, yield load, and the mode of failure. Stiffness was determined as the slope of the linear region of the load elongation curve.

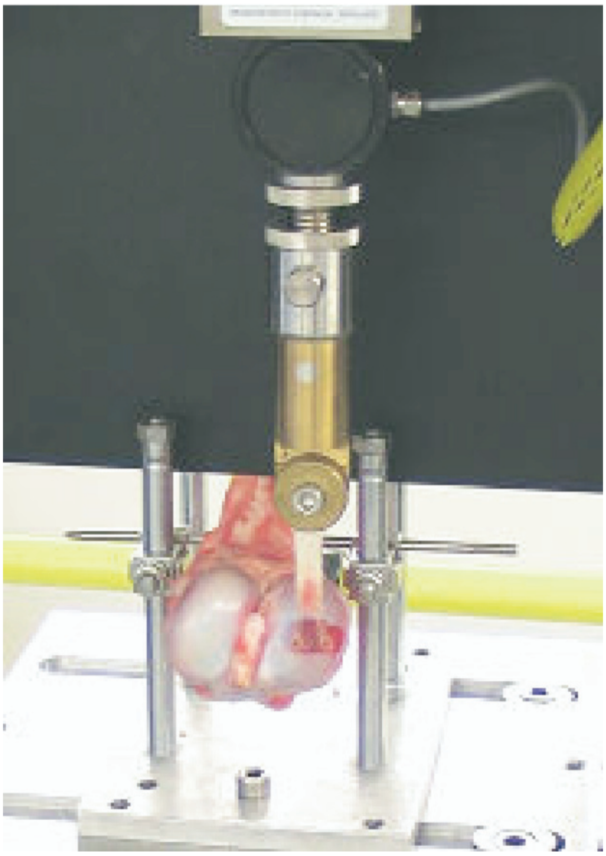

FIGURE 2. Test set up.

Statistics

The Kolmogorov–Smirnov test was used to test the normal distribution within the groups. A one-way analysis of variance (ANOVA) test was used to evaluate overall differences between the different test groups for load at failure and stiffness ($P < .001$).

When overall group differences were observed, the Scheffés procedure was used as a post-hoc test to identify the specific location of statistically significant differences (SPSS v 11.0; SPSS, Inc., Chicago, IL). Student *t* test was used when for differences within 1 subgroup (e.g., yield load and maximal load).

RESULTS

For all 3 parameters tested (yield load, ultimate load, and stiffness), the ANOVA revealed a group difference. Stiffness was 6.62 ± 0.60 N/mm in group 1;

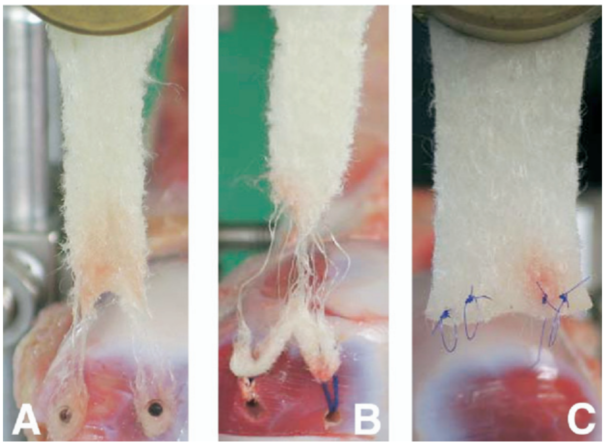

FIGURE 4. Typical examples of the failure mode of the tested fixation techniques: (A) failure mode of the pin fixation technique, (B) failure mode of the transosseous fixation technique, and (C) failure mode of conventional suture fixation technique.

3.83 ± 0.76 N/mm in group 2; and 1.62 ± 0.23 N/mm in group 3 (Fig 3A). The difference between group 1 and group 3 and between group 2 and group 3 was statistically significant ($P < .001$).

In group 1 (pin fixation), the mean yield load was 77.37 ± 11.86 N; in group 2 (transosseous suture technique), the mean yield load was 61.89 ± 10.71 N; and in group 3 (conventional suture technique), the mean yield load was 16.62 ± 5.35 N (Fig 3B). Mean yield load in group 1 and group 2 was significantly higher than in group 3 ($P < .001$).

Ultimate load was 88.56 ± 14.39 N in group 1; 72.30 ± 10.96 N in group 2; and 17.08 ± 5.32 N in group 3 (Fig 3C). The difference between group 1 and 2 and group 3 was statistically high significant ($P < .001$).

In group 1, all specimens failed by tearing the scaffold (Fig 4A). In group 2, the suture anchors of the scaffold were pulled out of the drill holes (Fig 4B). In group 3, the sutures cut through the uninjured cartilage (Fig 4C; Table 1).

DISCUSSION

The aim of the present study was to evaluate the structural properties of M-ACI during arthroscopic fixation using a transosseous suturing technique[2] and a technique using biodegradable pin devices,[10] and to compare the results to conventional suture techniques. Our hypothesis was that the yield load, maximal load, and stiffness of both arthroscopic fixation techniques are superior to these of conventional suture techniques. The results of the present study support our initial hypothesis. The pin fixation techniques and the transosseous fixation technique had significantly higher ultimate failure load, yield load, and stiffness than the conventional suture technique for the fixation of bioabsorbable scaffolds which can be used for autologous chondrocyte transplantation.

This surgical procedure is called M-ACI. The arthroscopic approach can minimize disadvantages of the open technique for ACI, such as adhesions, decreased range of motion, and scar formation.[2]

In the present study, a stiff, polymer-based matrix composed of polyglycolic acid fibers (Bioceed-C) was used.[11] The importance of a stiff scaffold on cellular viability has been illustrated by several authors.[12,13] The viability of the chondrocytes when using this matrix was demonstrated in several experimental studies.[14-16] These studies have also shown resorption of the scaffold, regeneration of the cartilage, and healing of the matrix to the surrounding cartilage.[14,15] Three-dimensional arrangement of human articular chondrocytes in resorbable polyglactin-polydioxanon fleeces supports chondrogenic differentiation and the formation of a hyaline-like cartilaginous matrix in vitro and in vivo.[16] A polymer-based matrix was also used by Erggelet et al.[2] for ACI.

Other scaffolds made of other biomaterials such as collagen (MACI [Verigen, Leverkusen, Germany], Novocart [Aesculap, Tuttlingen, Germany]), or hyaluronate (Hyalograft; Merz Pharma, Vienna, Austria) are in clinical use for autologous chondrocyte transplantation.[4-6] However, the clinical experience with all these scaffolds is limited, and only short-term results about open M-ACI have been published. These results were encouraging.[1]

The failure analysis shows that the material properties of the scaffold have a strong influence on the biomechanical tests. Therefore, the results of the present study cannot be transferred to the fixation of other matrices.

The exact forces to which the scaffold is subjected

TABLE 1. *Failure Mode*

Technique	Failure of the Scaffold	Pull out of Bone	Pull out of the Sutures Through the Cartilage
Pin fixation (n = 10)	10	—	—
Transosseous fixation (n = 10)	—	10	—
Conventional suture (n = 10)	—	—	10

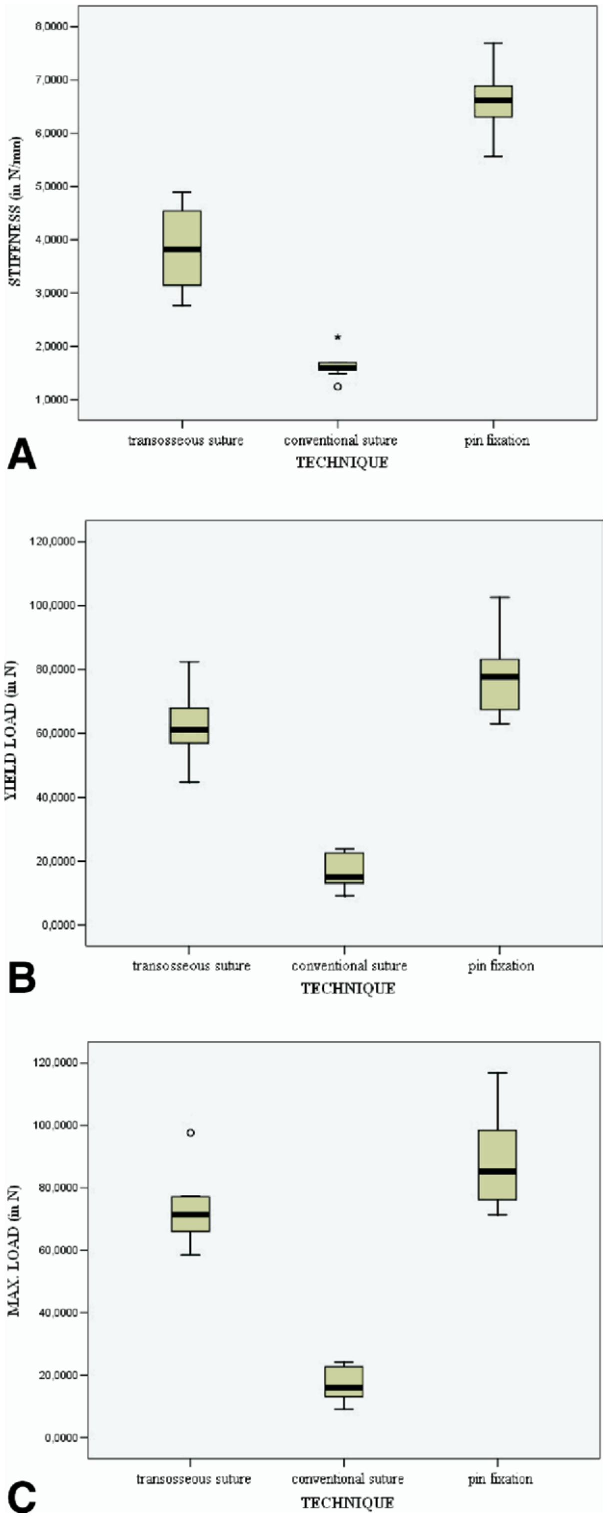

FIGURE 3. Box plots of structural properties: Stiffness (A), yield load (B), and ultimate failure load (C) of both arthroscopic fixation techniques are statistically significantly higher compared to the open conventional suture technique ($P < .001$).

in vivo are unknown. To answer this question, more research is needed. However, Peterson et al.[17] published long-term results of patients with isolated lesions at the femoral condyle treated after ACI. In this study, the periosteal flap was fixated with the conventional suture technique as tested in the present study. After 2 to 9 years, 92% of these patients have good to excellent clinical results.[17] In the present study, the failure mode of conventional sutures was cutting through the uninjured cartilage, and this technique had significantly lower yield load, ultimate failure load, and stiffness than both "arthroscopic" fixation techniques.

Current open techniques of fixation in M-ACI include the use of fibrin glue. This method was not tested in the present study because the arthroscopic fixation of a matrix seems to be a technical challenge.

Damage of the cartilage could be problematic when using the pin fixation technique. In a previous study in cadaveric bones, the surface of the construct seemed to be smooth.[10] However, when the pin is not properly implanted, the head becomes prominent, and there is a risk of cartilage damage at the opposite side. Further research is needed to evaluate if the pin has the potential to cause cartilage damage.

To our knowledge, the biomechanical characteristics of fixation techniques for biodegradable scaffolds for autologous chondrocyte transplantation have not been published. Therefore, it was not possible to adopt a well-examined test set-up for the present study. Because of the higher friction, the scaffold is subjected to shearing forces during movements of the joint. Therefore, in the present study, the test set-up was designed to imitate a shear force scenario.

A few limitations apply to this study. The study design does not reflect what really happens in a human knee. The lack of axial loading can considerably affect the results. During early rehabilitation, the scaffold is repetitively loaded during exercise or daily living activities, such as walking. Cyclic loading seems to duplicate the physiological loading conditions more closely than single-cycle failure tests. The results of the present study may serve as scientific basis for the development of cyclic loading protocols. In this study, we used a porcine model. It has been shown that the bone mineral density of porcine bone is comparable to that of the human femur.[18] Cadaver material of young donors in the typical age of patients who undergo ACI is difficult to obtain, and the variability in the bone mineral density of older body donors could lead to highly variable results.[18]

CONCLUSIONS

Our biomechanical data show that both fixation techniques—pin fixation and transosseous fixation—have a higher ultimate load, yield load, and stiffness than the conventional suture technique at time point zero. However, these data can be interpreted only with the Bioceed-C matrix.

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