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Mechanical testing of fixation techniques for scaffold-based tissue-engineered grafts

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KEYWORDS

ABSTRACT

Full-thickness defects in articular cartilage can be functionally restored by autologous chondrocyte implantation (ACI). In past years, numerous types of scaffolds for tissue-engineered cartilage implants have been developed and thoroughly characterized. However, the fixation stability of the implants has been rarely investigated despite its well-known importance for successful therapy. In this study, we have mechanically tested the fixation stability of four commonly used biomaterials for ACI attached by four different fixation techniques (unfixed, fibrin glue, chondral suture, and transosseous suture) *in situ*. Scaffolds based on polyglycolic acid (PGA) and polyglycolic acid and poly-**L**-lactic acid (PGLA), collagen membranes, and a gel-like matrix material were fixed within rectangular full-thickness cartilage defects of 10×15 mm^2 and loaded in tension until failure. Fibrin glue fixation of PGLA-scaffolds withstood a load of 2.18 6 ± 0.47 N, chondral sutured PGA-scaffolds of 26.29 6 ± 1.55 N, and transosseous fixed PGA-scaffolds of 38.18 6 ± 9.53 N. The PGA-scaffold could be loaded highest until failure for all fixation techniques compared to the PGLA-scaffold and collagen membrane. Our findings serve as basis for selecting the most suitable fixation technique for scaffold-based tissue-engineered grafts according to the expected *in vivo* loads. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 2007

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INTRODUCTION

Autologous chondrocyte implantation (ACI) is a promising technique to restore the functional property of articular cartilage in full-thickness defects. First clinical experiences with a suspension of cultured autologous chondrocytes beneath a sealed periosteal flap were published in 1994.[1] Since then, thousands of ACI were applied[2] and, meanwhile, surgical technique was improved by the replacement of the periosteal flap with biodegradable materials such as collagen sheets.[3] Alternatively to this cell-suspension approach, one aims at mimicking the natural *in vivo* environment of the chondrocytes with bioresorbable three-dimensional matrices for scaffold-based tissue engineering. Such scaffolds provide a better initial mechanical stability, homogeneous cell distribution, and improved tissue differentiation compared to two-dimensional systems.[4] Various natural[5][6] and synthetic[7] biomaterials have been shown to be suitable for scaffold-based cartilage tissue engineering and to promote repairing and resurfacing of articular cartilage defects. Their biochemical, histological, chondrogenic, immunohistochemical, and structural properties *in vitro* have been thoroughly investigated and optimized. However, for successful clinical application the tissue-engineered implants have to possess additionally an adequate stability to withstand handling during implantation as well as loading *invivo* and to enable a secure primary fixation as well as a stable and permanent integration into the surrounding tissues.

At present, the mechanical properties of *in vitro* cultured tissue-engineered cartilage implants and the unseeded scaffolds are about one order of magnitude lower than native articular cartilage.[8] However, these properties enable the surgical handling while implantation. Furthermore, a significant gain in mechanical quality can be observed *in vivo*.[9] The integration of the implant to the surrounding cartilage is dependent on the adjacent tissue architecture, its composition, and transport properties[10] and might be influenced by enzymatic treatment[11] and the developmental stage of the cartilage.[12] Furthermore, without a stable and enduring fixation of the implant, its integration into the surrounding tissue might not be achieved. If the fixation of the sample fails *in vivo* and the scaffold gets detached partially or even completely the loose body in the joint results in locking or catching of the knee[13] and consequently in poor clinical outcome.[14] In clinical practice cartilage transplants are fixed in open procedures or arthroscopically[15] into carefully debrided defects. For the cells suspension approach of ACI, the membrane material is sutured to the rim of the surrounding cartilage surface using and sealed with fibrin glue.[3] Three-dimensional matrices for scaffold-based tissue engineering are fixed in clinical practice either by press-fitting, by cartilage suture, by fibrin gluing, or by transosseous sutures to the subjacent bone.[15-17] Gel-like matrices are press-fitted into the defect.[18] In ACI, Driesang and Hunziker reported the delamination of the periosteal flaps in an animal study even after an immobilization time for 2-6 weeks.[19] Without immobilization, all flaps were detached. In a clinical study of matrix-induced ACI, two of 16 patients showed a partial or complete detachment of the graft fixed with fibrin glue in an early postoperative stage.[17]

The importance of the (primary) fixation of the tissue-engineered articular cartilage is well known,[20] but to date, only few studies exist that deal with fixation techniques and their mechanical properties. Recently, Drobnic and colleagues[21] reported the first systematic analysis of the stability of primary fixation for cartilage tissue-engineered grafts. They investigated different fixation techniques for collagen fleeces in a cadaveric study by continuous passive motion (CPM) of the tibiofemoral joint with and without load to simulate the initial postoperative period while rehabilitation. However, they focused on clinically relevant parameters such as incision length, operative time, and cost of the fixation material. The collagen scaffold fixation was only assessed qualitatively based on the integrity of the scaffold, the area coverage, and the manually tested fixation strength of the sample after CPM.

The aim of the present study was to quantify and compare the primary fixation stability of commonly used biomaterials for ACI attached by different fixation techniques *in vitro*. We tested two different synthetic three-dimensional scaffolds, a collagen-membrane and a gel-like matrix material representing the numerous commercially available biomaterials clinically used in cartilage repair. Since frictional forces are supposed to be a major reason for the delamination of the implants,[19] we simulated this loading regime by performing mechanically tensile test on the fixed scaffolds.

MATERIALS AND METHODS

Sample Preparation

Bovine patellas were obtained from the Institute of Veterinary Pathology at the University of Zurich and from the local abattoir and stored frozen at -20°C until the day of testing. After optical assessment of the articular cartilage, the surrounding tissue was removed and the patellas were embedded in acrylic resin (Beracryl, Suter-Swiss Composite Group, Fulenbach, Switzerland) to ensure rigid fixation during the test. To moisten the tissue during preparation and before testing the cartilage surface was covered with tissue paper soaked in phosphate-buffered saline (PBS). Rectangular (15×10 mm^2) full-thickness cartilage defects down to the subchondral bone were cut using a scalpel on the lateral facet of the proximal patella. The thickness of the surrounding cartilage rim varied between 1.6 and 3.9 mm.

Preparation and Fixation of the Scaffolds

Since the biomechanical properties of tissue-engineered grafts depend initially in the post-operative stage on the mechanical properties of the scaffold material, all tests were performed without cultured chondrocytes. Besides, the materials were soaked in PBS and treated according to the instructions of the manufacturer. A fleece consisting of polyglycolic acid (PGA) and poly-L-lactic acid (PLLA) in a 90:10 ratio and small amounts of polydioxanon (Ethisorb, Ethicon, Norderstedt, Germany) (PGLA) with a thickness of 2.2 mm was soaked in a human fibrinogen-solution [33% (v/v) (Tissucol, Baxter, Heidelberg, Germany) in Ham's F12 medium (Biochrom, Germany) containing 10% human serum]. Fibrinogen was polymerized by adding thrombin (1:10 in PBS, Tissucol, Baxter, Germany) and stored in PBS for 2 days after fabrication. The PGA fleece (alphaResearch Switzerland GmbH, Germany) with a thickness of 1.1 mm was conditioned in PBS for 5 min prior to fixation. The collagen membrane (BioGide, Geistlich Biomaterials, Wolhusen, Switzerland) was sutured dry and soaked in PBS for 5 min prior to testing according to clinical practice. The BD Matrigel^{TM} Basement Membrane Matrix (BD Biosciences, Bedford, MA, USA), a representative of the gel-like matrices, was thawed and transferred into the defect with a spatula. We tested the PGLA- and PGA-scaffolds unfixed, fixed with fibrin glue, and fixed with transosseous and chondral suture. Collagen membranes were fixed with all but the transosseous suture, since this method is clinically not applied. The Matrigel was only tested unfixed for the same reason. An overview of the scaffolds and the fixation techniques used in this study is given in Table I.

Table I. Summary of the Tested Combinations of Scaffolds With the Fixation Techniques

	Type of Fixation			
Scaffold Material	Unfixed (UF)	Fibrin Glue (FG)	Transosseous Suture (OS)	Chondral Suture (CS)
PGLA-scaffold	X	X	X	X
PGA-scaffold	X	X	X	X
Collagen Membrane	X	X	c.n.a.	X
BD Matrigel^{TM} Matrix	X	c.n.a.	c.n.a.	c.n.a.

c.n.a., clinically not applied.

For the unfixed testing (UF) the scaffold was placed directly onto the subchondral bone without additional material.

For the fixation with fibrin (FG), the fleece and the membrane were fixed into the clamping mechanism prior to testing. We applied 0.2 mL of the two-component fibrin sealant (Tissucol Duo, Baxter, Heidelberg, Germany) onto the subchondral bone and the surrounding cartilage rim of the cartilage defect. The clamped samples were pressed into the defect for 4 min according to the manufacture recommendation and clinical practice and fixed to the testing machine.

Cartilage-suture fixation (CS) was performed according to clinical practice.[1][22] The scaffold was placed into the defect and sutured to the cartilage rim with eight interrupted stitches (PDS 6-0, Ethicon, Johnson&Johnson, Germany) at a distance of approximately 4 mm from each other.

Transosseous suture (OS) fixation was performed in all four corners of the cartilage defect as described previously.[15] However, we had to drill the holes using a conventional drill bit (1.5 mm in diameter) before using the Kirschner-wires (1.7 mm in diameter) to expand the holes since the acrylic resin was too strong and ductile for the guide wires. The scaffolds were armed on their corners by means of a resorbable thread (Vicryl 2-0, Ethicon) with a three-fold knot to secure the sling and an additional sling to pull the scaffold into the defect. The knots were guided into the drilled holes by pulling firmly on the additional sling. Thus, scaffold is anchored in the bone by press-fitting of the knots.

Mechanical Testing

To assure reproducible fixation of the scaffolds the samples were clamped between two defined rough surfaces [Figure 1(C)]. Using a spacer between the bottom and top holder the PGA- and PGLA scaffolds were clamped with approximately 20% strain.
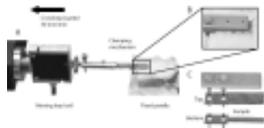
Figure 1. Image of the mechanical testing setup with the moving crosshead, the fixed patellar, and the clamping mechanism to attach the sample to the load cell (A). Enlarged section (B) and exploded view (C) of the clamping mechanism with the sample between bottom and top holder.
[Normal View 25K | Magnified View 80K]

Uniaxial tensile tests of the fixed scaffolds were performed parallel to the subchondral bone using a Zwick material testing machine (Zwick Z2.5, Zwick, Ulm, Germany) with a 50 N load cell (KAP-S, Angewandte System Technik AST, Wolnzach, Germany). The patellar samples were transferred to the mechanical testing machine and the subchondral bone surface was aligned to the direction of the machine axis. The fixed samples were loaded with a crosshead speed of 50 mm/min [Figure 1(A,B)]. To characterize the fixation stability of the scaffolds, the ultimate tensile load and the type of failure were assessed. Failure of the sample was defined as abrupt decrease of the resisting load by 20 N/s. Failure can result in complete loss, partial detachment, or slight rupture of the scaffold or the fixation. To test the failure behavior of the collagen membrane fixed by chondral suture [Figure 2(C)] and the transosseous fixed PGA scaffold [Figure 2(I)], the aforementioned clamping mechanism was improved by a surgery clamp [Figure 2(D,J)].
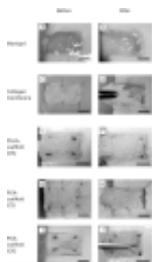
Figure 2. Exemplary macroscopic views of the type of failure for the Matrigel (first row), collagen membrane (second row), transosseous fixed (OS) PGLA scaffold (third row), chondral (CS) (fourth row), and transosseous fixed (OS) PGA scaffold (fifth row). First column shows the initial configuration before testing, and second column the appearance after testing. Chondral fixed PGLA-scaffold is not shown (type of failure analog to PGLA (OS)). The surgery clip for improved clamping can be seen in D and J. The artificial defect with a size of 10 mm × 15 mm is open on the left side. Scale bars, 5 mm.
[Normal View 38K | Magnified View 139K]

Statistical Analysis

Maximal tensile load is displayed as mean ± standard deviation (SD) for five samples per group unless otherwise noted. To examine the effect of the fixation technique on the tensile load for the collagen, PGA- and PGLA scaffold, the data were analyzed statistically using an analysis of variance (ANOVA), followed by a *post-hoc* assessment applying the Tukey's HSD method. A two-way ANOVA was performed to examine the effect of the material and the fixation method on the maximal tensile load for all data in Systat (Systat Software, Point Richmond, CA, USA). Differences were considered significant at $p < 0.05$.

RESULTS

The bottom holder by itself glued to the subchondral bone without a fixed scaffold withstood the crosshead movement up to a load of 0.18 ± 0.08 N ($n = 3$). The clamping mechanism was capable of fixing the synthetic PGA and PGLA scaffolds up to a load of 28.55 ± 10.11 N and for the collagen membrane to a load of 5.35 ± 1.85 N, whereas the surgery clamp enabled fixation until failure.

The maximal load of the unfixed samples was about 0.1 N for all materials. Two-way ANOVA revealed that the PGA-scaffold could be loaded highest (21.8 N) until failure for all fixation techniques compared to the collagen membrane (16.1 N) and the PGLA scaffold (10.4 N). Considering the fixation technique as only parameter of the

maximal tolerated load, the two-way ANOVA revealed higher load for transosseous fixation (30.16 N) compared to chondral suture (16.85 N) and fibrin glue fixation (1.69 N). Highest failure loads for the fibrin glue fixation can be obtained using PGLA scaffolds (2.18 ± 0.47 N), whereas significantly higher failure loads (26.29 ± 1.55 N) for chondral sutures can be obtained using PGA scaffolds (Figure 3). At this loading range the sutures were torn out from the surrounding articular cartilage [Figure 2(H)].
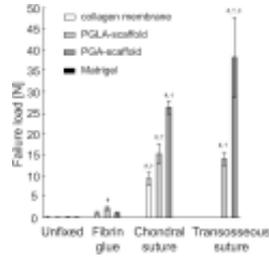
Figure 3. Plot of the maximal tensile loads for the unfixed, fibrin glue fixation, chondral suture fixation, and transosseous suture fixation of the collagen membrane, PGLA-scaffolds, and PGA-scaffolds. Maximal loads are given as mean and error bars represent SD. # indicates significant differences to unfixed sample, † significant differences to fibrin glue fixation, and ‡ significant differences to chondral suture ($p < 0.05$, ANOVA). [Normal View 24K | Magnified View 58K]

The knots of the transosseous fixed PGA-scaffolds were torn out from the subchondral bone [Figure 2(J)] at significantly higher load of 38.18 ± 9.53 N (Table II).

Table 2. Summary of the Maximal Tensile Loads and the Type of Failure for All Tested Combinations of Scaffolds and Fixation Techniques

| Scaffold Material	Type of Fixation	Unfixed (UF)	Fibrin Glue (FG)	Transosseous Suture (OS)
Matrigel	Unfixed	4	~0.1	Failure of gel [Figure 2(B)]
Collagen Membrane	Unfixed	4	0.08 ± 0.03	
	Fibrin glue	5	1.00 ± 0.44	Fibrin glue adhesion at subchondral bone
	Chondral suture	4	9.29 ± 1.58[#,†]	Membrane [Figure 2(D)]
PGLA Scaffold	Unfixed	3	0.09 ± 0.01	
	Fibrin glue	5	2.18 ± 0.47[#]	Fibrin glue adhesion at subchondral bone
	Chondral suture	5	15.21 ± 2.35[#,†]	Rupture of scaffold
	Transosseous suture	5	13.96 ± 1.61[#,†]	Rupture of scaffold [Figure 2(F)]
PGA Scaffold	Unfixed	3	0.11 ± 0.07	
	Fibrin glue	5	1.07 ± 0.31	Fibrin glue adhesion at subchondral bone
	Chondral suture	4	26.29 ± 1.55[#,†]	Suture in cartilage [Figure 2(H)]
	Transosseous suture	4	38.18 ± 9.53[#,†,‡]	Transosseous fixation [Figure 2(J)]

Data represent mean ± SD for *n* = 4-6.

\# indicates significant differences to unfixed sample.

† significant differences to fibrin glue fixation.

‡ significant differences to chondral suture ($p < 0.05$).

More precise analysis shows that fibrin glued PGA- and PGLA-scaffolds withstood a load of 1.07 ± 0.31 N and 2.18 ± 0.47 N, respectively, and the glued collagen membrane resisted a force of 1.00 ± 0.44 N (Table II). All these combinations failed at the adhesion site of fibrin glue to the subchondral bone with a complete loss of the matrix material from the defect. Chondral suture failed in the surrounding articular cartilage at a maximal load of 26.29 ± 1.55 N (Table II). All other chondral-sutured combinations failed by partial detachment within the scaffold at a force of 9.29 ± 1.58 N and 15.21 ± 2.35 N for the collagen membrane and the PGLA-fleece, respectively. The transosseous fixed fleece scaffolds failed partially because of rupture of the clamping mechanism for the PGLA scaffold at 13.96 ± 1.61 N and because of the bone fixation failure for the PGA scaffold (38.18 ± 9.53 N). The resisting load of the Matrigel against quasi-static loading was in the range of the blank test of the clamp mechanism against the subchondral bone (0.10 ± 0.05 N). All failure loads are visualized in Figure 3 and statistics and descriptions of the type of failure are given in Table II.

Typical load-curves for the transosseous sutured, the chondral sutured, the fibrin glued, and the unfixed samples were shown exemplarily for the PGA-scaffolds in Figure 4. Transosseous suture appears to be stiffer than cartilage suture.
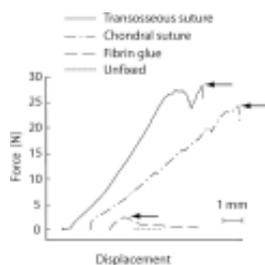
Figure 4. Example of a typical load-displacement curve for the PGA scaffold fixed by transosseous suture (solid line), cartilage suture (dash dotted line), fibrin glue (coarse dashed line), and unfixed (fine dashed line). Arrows indicate maximal tensile load. Scale bars, 1 mm.
[Normal View 22K | Magnified View 51K]

DISCUSSION

In the present study we assessed the primary fixation stability of a variety of clinically relevant, scaffold-based tissue-engineered grafts for full-thickness cartilage defects under tensile loads *in situ* on bovine patellas to simulate shear stress. Since the biomechanical properties of commonly used scaffold-based autologous chondrocyte grafts are initially mediated by the respective biomaterial, we loaded the biomaterials quasi-statically until failure and assessed the loads at failure as well as the type of failure of the scaffold-fixation combination. Comparing the different fixation techniques and scaffold materials revealed that PGA scaffolds withstood the highest loads independent of the type of fixation used. Regarding the fixation techniques independent of the scaffolds use in this study, we showed that the transosseous fixation withstood higher load than the chondral suture, the fibrin glue fixation, and the unfixed positioning.

In healthy synovial joints the frictional coefficient and thus the frictional force at the cartilage-cartilage interface is very low with a dynamic frictional coefficients of about 0.02.[23] However, cartilage-cartilage friction might be increased significantly with loading time,[23] by experimental scouring of the cartilage surface,[24] or by chemical degradation of the cartilage surface simulating OA-like changes.[25] For engineered cartilage samples the transient frictional coefficient against a flat steel-plate might be even up to five times higher than native cartilage due to exudation of interstitial water[26] and thus due to the reduction of lubrication. Reaction force of about 560 N might occur within a patellofemoral joint of a subject of 75 kg during free walking[27] or of about 2200 N during stair descent.[28] With a low frictional coefficient of about 0.02, this loading *in vivo* might result in shear loads of 11 N while free walking or of 44 N while stair descent. These superficial *in vivo* shear loads are in the range of the failure loads shown in this study. However, a roughened subchondral bone might fix the sample additionally *in vivo* due to frictional forces and form locking between scaffold and bone. The presently used test-design excludes this additional fixation of the scaffold to the bone due to the less rough subchondral bone surface of the patellar bone compared to the tibial or femoral bone observed in clinical practice. This allows for the reproducible assessment and comparison of the stability of the fixation techniques independent of the hardly controllable surface roughness. As a result, the fixation strength *in vivo* may be enhanced compared to these standardized conditions.

Fixation technique without any anchorage of the sample (unfixed) is dependent on the press fitting of the sample into the defect and the roughness of the subchondral bone. Press fitting *in vivo* may be increased due to the compression and consequently the lateral expansion of the scaffold. Nevertheless, Drobnic et al.[21] also observed a least stable fixation of press-fitted scaffold in their *in situ* cadaveric study. Therein, the press-fitted scaffolds were detached before 60 cycles of unloaded CPM.

The fibrin glue fixation techniques in our study withstood tensile loads between 1 and 2.18 N, which presumably corresponds to the detachment "with minor pull" in the study of Drobnic et al.[21] Although the difference between unfixed and fibrin glued samples was only statistically significant for the PGLA scaffold, fibrin glue fixation improves the maximal tolerable load until failure by a factor of 10-20 compared to unfixed scaffolds. Drobnic et al.[21] reported that loading of the knee with 10 and 20 kg and performing passive motion resulted in detachment of the glued scaffolds. With an assumed frictional coefficient of 0.02 between femoral cartilage and scaffold, this load results in frictional shear forces of approximately 2 and 4 N on the surface of the scaffold, respectively. Standardization of these loads with the reported glued defect area of 250 mm^2 results in an overall maximal shear stress at the scaffold-bone interface which is comparable to our *in vitro* results for the fibrin-soaked PGLA fleeces of 13 kPa (area = 150 mm^2). We observed that the fibrin glue fails at the adhesion site at the subchondral bone for all materials. As the resisting load of the glued bottom holder (0.18 ± 0.08 N) is slightly higher than the frictional force between the bottom holder of the clamping mechanism and the subchondral bone (0.10 ± 0.05 N), we concluded that the gluing area is not reduced by the bottom sample holder. Consequently, the entire defect size of 150 mm^2 can be used for stress calculation. Maximal shear stress at the interface of the patellar subchondral bone and the fibrin glue, calculated from the collagen membrane data where the additional fixation at the surrounding cartilage rim can be neglected, is about 6.7 kPa. The difference between the maximal load of the PGLA- and the PGA-scaffold of approximately 1 N can be explained by an additional shear and tensile strength of the fibrin glue-cartilage interface at the cartilage rim (area = 40 mm^2) of 25 kPa, which is comparable to the literature values of approximately 20 kPa after 5 min of incubation.[29]

Cartilage suture fixation withstood the loads of 26.29 ± 1.55 N when the suture cut through the healthy cartilage tissue [Figure 2(H)]. One might expect this maximal load to be even more reduced *in vivo* due to degenerative disruption of the circumjacent tissue. Transosseous fixation was the most stable fixation method for the 3D fleece materials in this test. The failure load was the highest of all techniques with 38 N, even for the aggravated test procedure using the surgery clip. The cartilage suture fixation of PGA-scaffolds is slightly softer under tensile loads due to the elastic properties of the surrounding cartilage tissue and the thinner suture thread.

The PBS-soaked collagen membranes display a very low friction that complicated the fixation within the clamping mechanism for testing. However, the low friction makes it even more suitable for *in vivo* application due to resulting lower shear stress, which is likely to be responsible for the detachment of the scaffold. Furthermore, collagen membranes and other scaffolds, which are thinner than the surrounding tissue, are shielded from superficial shear loads by the surrounding cartilage. This might improve on one hand the fixation stability *in vivo* but on the other hand results in higher stresses of the surrounding cartilage rim, which could negatively effect cell survival[30] and consequently on graft integration.

Limitation of the study is the "open" side of the defect (Figure 2) that results in decreased scaffold press fitting and reduced surrounding cartilage area for fixation. However, compared to the clinical situation, the synthetically generated defect was very well defined and surrounded by healthy cartilage, which might improve press fitting compared to *in vivo* conditions. For the mechanical testing we reduced the complex *in vivo* stress conditions acting on biomaterials in articulating joints to mere shear stress and consequently to tension load acting on the scaffold. The compressive loads, increasing the press-fitting of the scaffolds as well as the frictional- and form locking of the scaffold with the underlying subchondral bone occurring *in vivo*, were neglected. These properties are likely to increase the failure load especially for the porous 3D-scaffolds. The clamping mechanism itself might have additionally an influence on the determined results due to high local stresses at the material. Thus, the failure loads of the transosseous and the chondral fixed PGLA-scaffold *in vivo* might be even higher due to the more moderate load-transmission. Furthermore, the Matrigel cannot be fixed reliably between the clamping mechanism due to the gel-like viscous property.

Despite these differences from the *in vivo* conditions we can conclude that the transosseous fixation fails partially at tensile loads of 38.18 ± 9.53 N and the chondral fails partially at tensile loads of 26.29 ± 1.55 N within the surrounding cartilage. The fibrin glue fixation gets completely detached at loads of 2.18 ± 0.47 N due to failure of the fibrin adhesion at the subchondral bone. Unfixed samples withstood smaller loads of 0.1 N. In general, taking these simplifications into account, the test design enabled us to test commonly used biomaterials for ACI reproducibly and to obtain and compare quantitative data of the primary-fixation stability for the first time. Thus, this testing method serves as a first step to improve the primary fixation and consequently the integration of tissue-engineered grafts. The herein presented data may advance the clinical specifications for the implantation, such as defect size, location of the defect, or the degenerative stage of the remaining tissue, and allows choosing

a stable fixation technique according to the scaffold for expected *in vivo* loads.

In the current study, we have assessed the fixation stability of four commonly used biomaterials for ACI attached by four different fixation techniques. We showed that the transosseous fixation withstood higher load than the chondral suture, the fibrin glue fixation and the unfixed positioning for all tested materials. Our findings of the quantitative comparison of the fixed scaffolds serve as a basis for selecting the most suitable fixation technique according to the expected *in vivo* shear loads on the implant.

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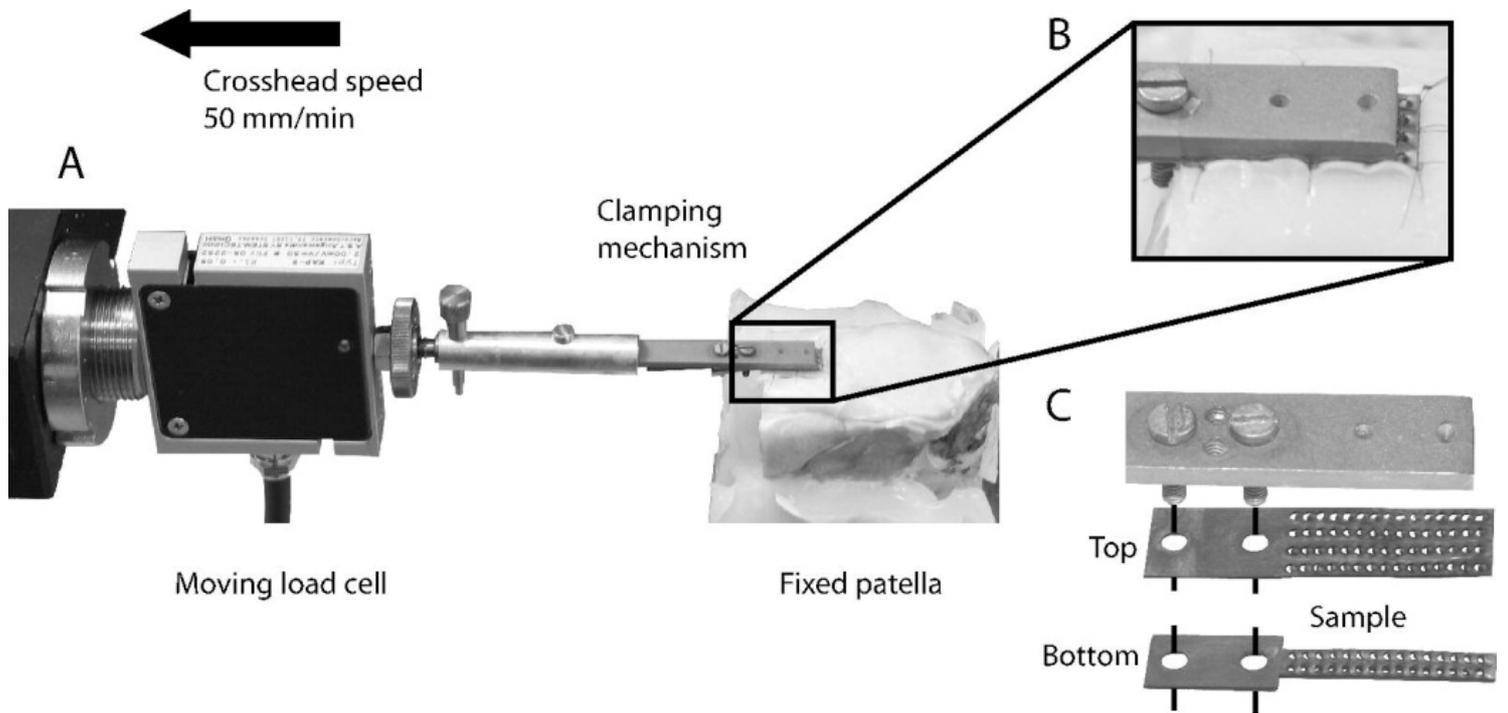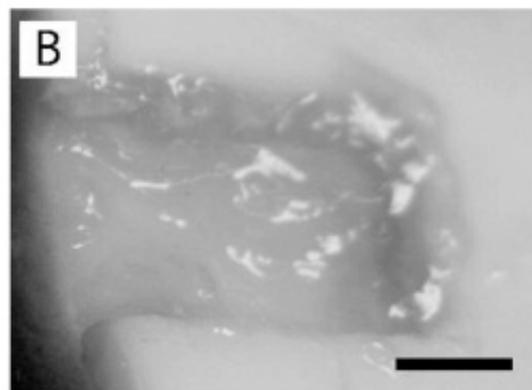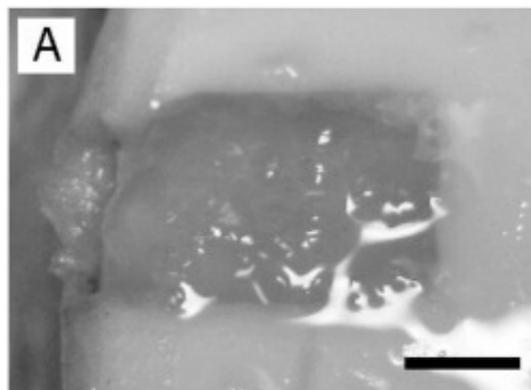

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Figure 1. Image of the mechanical testing setup with the moving crosshead, the fixed patellar, and the clamping mechanism to attach the sample to the load cell (A). Enlarged section (B) and exploded view (C) of the clamping mechanism with the sample between bottom and top holder.

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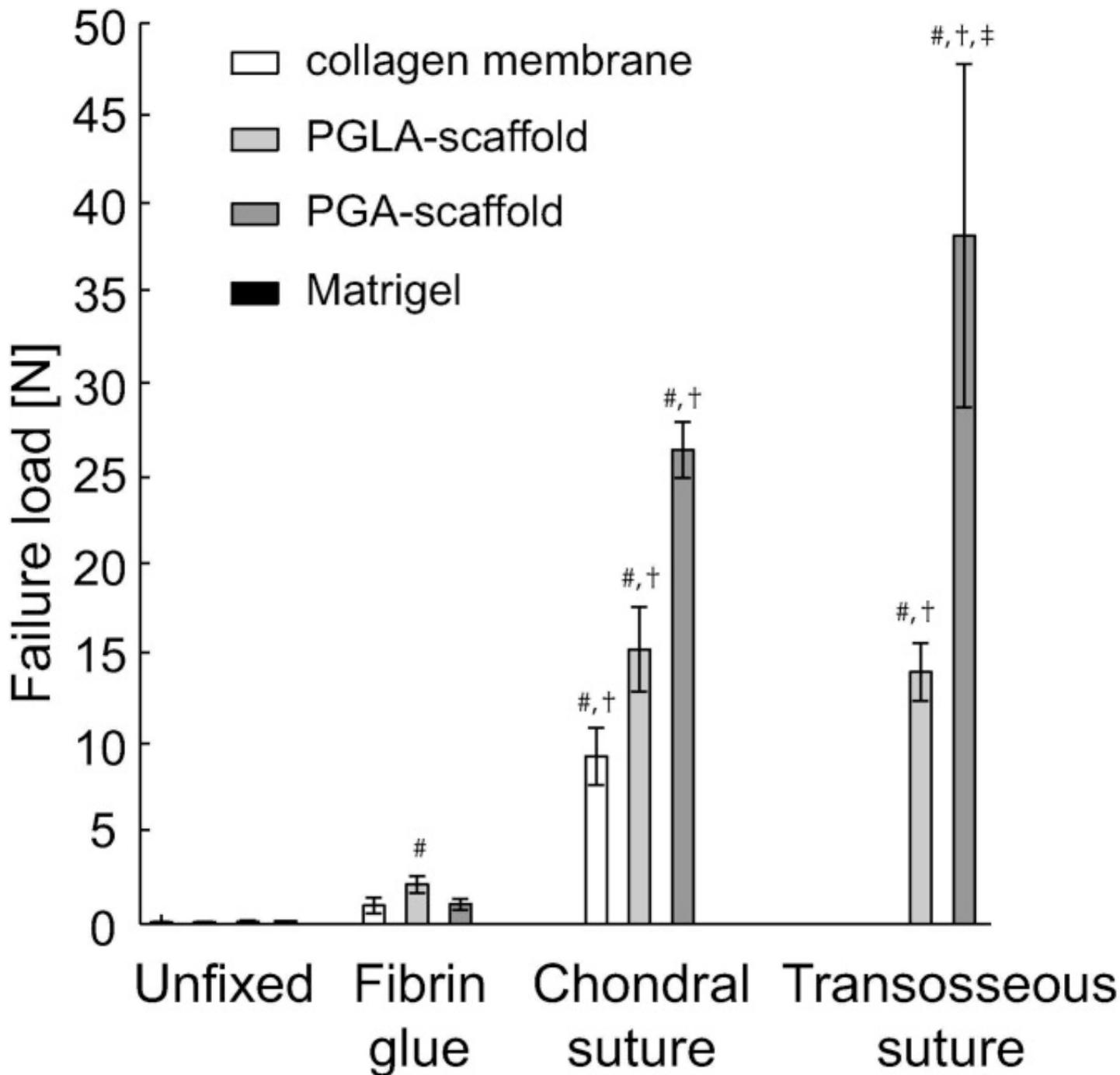(OS)

Figure 2. Exemplary macroscopic views of the type of failure for the Matrigel (first row), collagen membrane (second row), transosseous fixed (OS) PGLA scaffold (third row), chondral (CS) (fourth row), and transosseous fixed (OS) PGA scaffold (fifth row). First column shows the initial configuration before testing, and second column the appearance after testing. Chondral fixed PGLA-scaffold is not shown (type of failure analog to PGLA (OS)). The surgery clip for improved clamping can be seen in D and J. The artificial defect with a size of 10 mm × 15 mm is open on the left side. Scale bars, 5 mm.
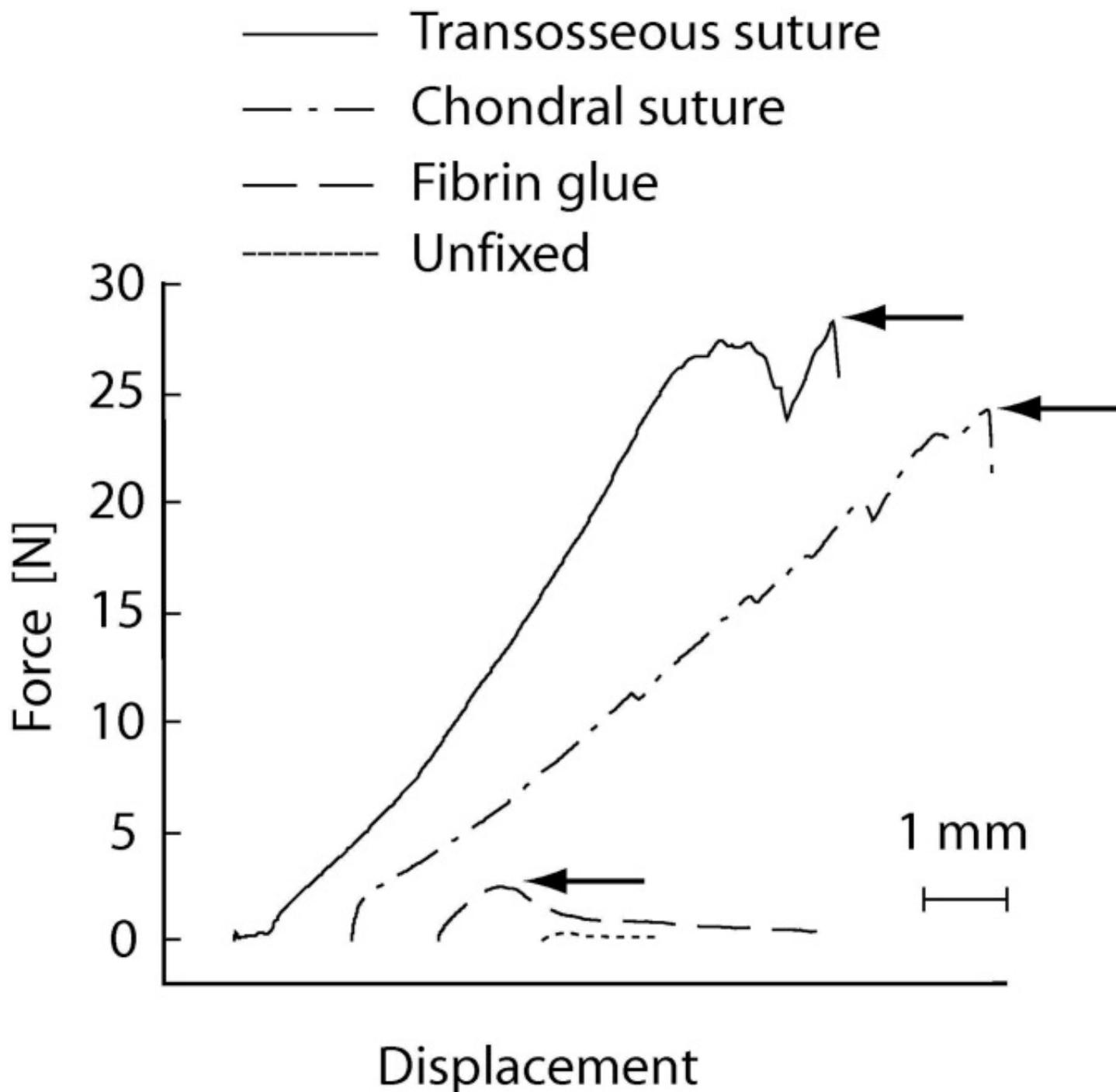
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Figure 3. Plot of the maximal tensile loads for the unfixed, fibrin glue fixation, chondral suture fixation, and transosseous suture fixation of the collagen membrane, PGLA-scaffolds, and PGA-scaffolds. Maximal loads are given as mean and error bars represent SD. # indicates significant differences to unfixed sample, † significant differences to fibrin glue fixation, and ‡ significant differences to chondral suture ($p < 0.05$, ANOVA).

Figure 4. Example of a typical load-displacement curve for the PGA scaffold fixed by transosseous suture (solid line), cartilage suture (dash dotted line), fibrin glue (coarse dashed line), and unfixed (fine dashed line). Arrows indicate maximal tensile load. Scale bars, 1 mm.