

Comparison of clinically used bilayer collagen membrane and trilayer collagen prototype fixation stability in chondral defects at the talus – An experimental human specimen study

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ABSTRACT

Background: The purpose of this human specimen experimental study was to compare the fixation stability of clinically used bilayer collagen membrane with fibrin glue with trilayer collagen prototype without fibrin glue in chondral defects at the medial or lateral talar shoulder (both matrices from Geistlich Pharma AG, Wollhusen, Switzerland).

Methods: Eleven human specimens were used. The membranes were implanted in standardized chondral defects at the medial and lateral talar shoulder randomized. All tests were performed in load-control 15 kg. Range of motion ROM of each ankle was examined individually before testing. The average ROM was 10° dorsiflexion range 0°–20° and 30° plantarflexion range 20°–45°. 1,000 testing cycles with the defined ROM were performed. Two independent investigators, blinded to membrane and fixation type, visually assessed the membrane fixation integrity for peripheral detachment, area of defect uncovered, membrane constitution and delamination.

Results: The clinically used bilayer collagen membrane plus fibrin glue showed higher fixation stability than the trilayer prototype (all $p < 0.05$). No significant differences occurred between medial and lateral talar shoulder location (all $p > 0.05$).

Conclusions: The fixation stability of the trilayer collagen prototype without fibrin glue is lower than of the clinically used bilayer membrane with fibrin glue in chondral defects at the medial and lateral talar shoulder in an experimental human specimen test. Clinical use of trilayer collagen prototype without fibrin glue has to be validated by clinical testing to evaluate if the lower stability of fixation is still sufficient.

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1. Introduction

Cartilage restoration at the talus for cartilage defects has been shown to be clinically successful with matrix associated stem cell transplantation (MAST) and autologous matrix induced chondrogenesis (AMIC) [1–12]. Radiologically complete filling of the defect was seen in 88% and in 52% hypertrophy of the cartilage layer after a mean follow up of 4.7 years was shown. But clinical results demonstrated significant pain reduction, recovery of ankle

function, and successful return to sport [4]. Especially with MAST, very favourable results up to five years of clinical follow-up have been published [9–11,13]. In a clinical matched-pair-analysis MAST was compared with AMIC with Peripheral Blood concentrate (PBC), and MAST and AMIC + PBC showed similar results [14]. For all three methods (MAST, AMIC, AMIC + PBC), the used matrix respectively membrane (Chondro-Gide, Geistlich Pharma AG, Wollhusen, Switzerland) is typically fixed with fibrin glue [1,10,11,14]. Partial autologous fibrin glue and collagen I/III matrices are favourable in respect to migration pattern, morphology and viability [15]. However, fibrin glue is a blood product which is expensive and may carry a risk of infection [11]. Therefore, a fibrin glue free fixation of the membrane is desired [11]. The purpose of this human specimen experimental study was to compare the fixation stability of clinically used bilayer collagen membrane with fibrin glue (Chondro-Gide) with trilayer collagen prototype without

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fibrin glue in chondral defects at the medial or lateral talar shoulder.

2. Methods

2.1. Membranes

Clinically used bilayer membrane (Fig. 1)

The bilayer membrane is a collagen I/III membrane (Chondro-Gide, Geistlich Pharma AG, Wollhusen, Switzerland) (Fig. 1). The first layer is the so-called cell-occlusive superficial layer, which is directed towards the joint (gap) (Fig. 1). This layer is designed to protect the tissue from shear forces and to prevent the cells from penetrating into the joint space. The second layer is the so-called porous layer, which is directed towards the subchondral bone (Fig. 1). This layer is designed to contain the cells. The cells are either from bone marrow aspirate concentrate (BMAC) when performed as MAST, and/or from the subchondral bone when microfracturing is additionally performed during MAST/AMIC.

Trilayer prototype (Fig. 2)

The trilayer collagen prototype is an experimental product with specially designed an extra layer directed towards the subchondral bone (Fig. 2). This third layer could potentially provide better adherence of the trilayer prototype to the subchondral bone.

2.2. Specimens

Eleven human specimens were used for the tests. Six were right sided and five left sided, six were male and five female specimens. The specimens included the entire foot and ankle and the distal part of the lower leg, i.e. up to 30 cm above the ankle level. The specimens were fresh-frozen without embalment. Relevant degenerative changes and/or deformities were considered as exclusion criteria for the specimen. For one specimen (No. 3, left, female) severe degenerative changes were detected by investigation including fluoroscopic imaging. Therefore, the specimen No. 3 was replaced with No. 11. For specimens No. 1–2 and 4–11 no relevant degenerative changes and/or deformities were detected by investigation including fluoroscopic imaging. Before testing, the specimens were thawed for 48 h in 20 °C environment. An antero-medial and antero-lateral approach were simultaneously performed as previously described [11]. The medial and lateral talar shoulder was exposed. The ankle was moved to maximum (up to 60°) plantarflexion which was measured with a goniometer (Electronic goniometer, Fix-Kit, Bucharest, Romania). This amount of plantarflexion was achieved with two assistants holding lower leg and foot and applying high torque. The cartilage defect was

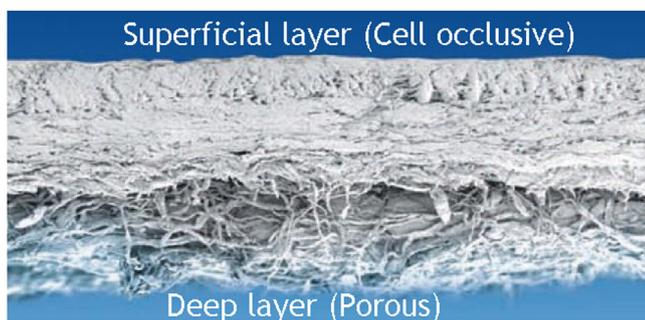


Fig. 1. Chondro-Gide membrane (Geistlich Pharma AG, Wollhusen, Switzerland). The first layer is the so-called cell-occlusive superficial layer which is directed towards the joint (gap). This layer is designed to prevent the cells from penetrating into the joint. The second layer is the so-called porous deep layer which is directed towards the subchondral bone. This layer is designed to contain the cells. Different sizes are available. Scaling is in cm.

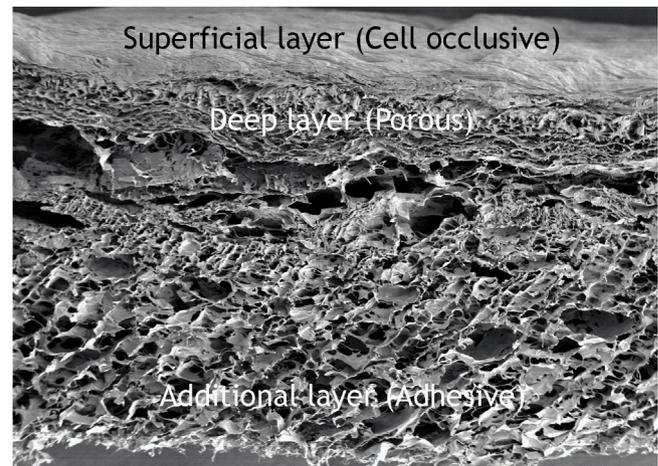


Fig. 2. Trilayer membrane (Geistlich Pharma AG, Wollhusen, Switzerland). The trilayer membrane contains the bilayer membrane with an extra added layer at the bottom, i.e. directed towards the subchondral bone. This third layer provides adherence of the trilayer scaffold to the subchondral bone.

created with a scalpel (15 blade, Aeskulap AG, Tuttlingen, Germany). The defect was exactly located at the medial and lateral talar shoulder including the talar shoulder itself and directed to the talar body. The location was exactly at the anterior margin of the tibia during 60° plantarflexion as described above. The cartilage defect was created with a square area of 1 cm × 1 cm (Fig. 3a). For this purpose, a template was used (Custom made). The cuts through the cartilage were perpendicular to the surface down to the subchondral bone. The cartilage was removed with a sharp spoon and microfracturing was performed with a 1.6 mm K-wire as previously described (Fig. 3a) [11].

2.3. Membrane implantation

Both membranes were immersed for 3 min in 0.9% NaCl-solution. Then they were cut to fit exactly in the chondral defects. The bilayer membrane was fixed in the chondral defect with fibrin glue (Tisseel, Baxter International, Deerfield, IL, USA) as described (Fig. 3b) [11]. The trilayer collagen prototype was inserted in the chondral defect without fibrin glue (Fig. 3c). After membrane implantation, the ankle was reduced to neutral position under manual traction. The capsule was closed with several single/interrupted sutures and the skin with one suture.

2.4. Stress-test

The specimens were tested as described before (Fig. 4) [16].

The foot and the proximal 170 mm of the remaining tibia were each potted with the use of bone cement (RenCast FC 52/53, Goessl + Pfaff, Karlskron, Germany) in an aluminium casing [16] before implementation of the matrices. A 2.0 mm K-Wire was implemented in the calcaneus transversely to enhance the stability of the embedding of the foot. After surgery, the foot was mounted to the test machine. Adjustment of the testing machine and standardized distance between the actuator and tibial axis was verified using a cross table [16]. Testing was commenced as soon as the resin had cured [16]. Measurements were performed on a biaxial test machine (Instron 8874; Instron, Darmstadt, Germany) equipped with a 10 kN/100 Nm load cell for compression, extension and torsion [16]. All tests were performed in load-control (15 kg simulated partial weight-bearing) [16]. Range of motion ROM for testing of each ankle was specified individually in the testing apparatus before testing. The average ROM was 10°

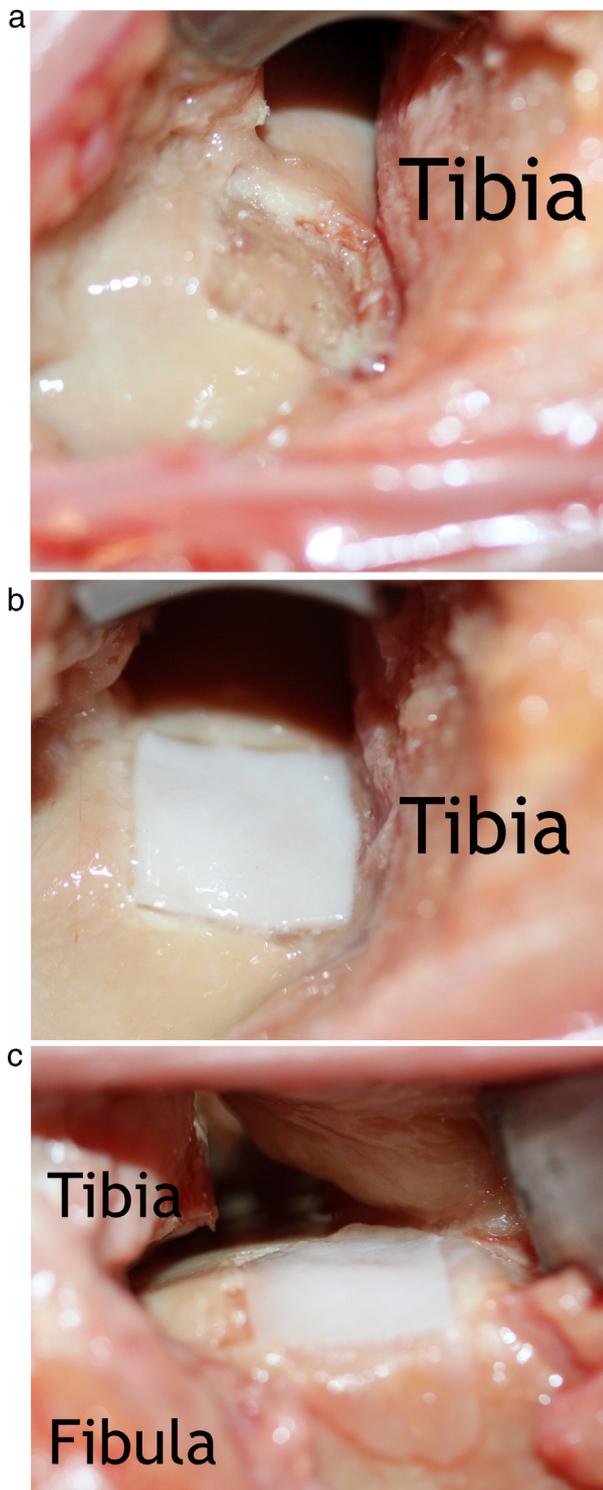


Fig. 3. (a–c) (a) Shows the chondral defect preparation at the medial talar shoulder. The cuts through the cartilage were perpendicular to the surface down to the subchondral bone. The cartilage was removed with a sharp spoon as described. A micro fracturing was performed with a 1.6 mm K-wire as described. (b) Shows the bilayer membrane in the same defect as Fig. 3(a) after testing with the following average scores: peripheral detachment, 0; area of defect uncovered, 0; patch constitution, 0.5; delamination membrane, 0. (c) Shows the trilayer membrane at the lateral talar shoulder after testing with the following average scores: peripheral detachment, 0.5; area of defect uncovered, 0.5; patch constitution, 0; delamination membrane, 0.

dorsiflexion range 0°–20° and 30° plantarflexion range 20°–45°. For each specimen, 1,000 cycles with the maximum ROM were performed.

2.5. Analysis of fixation stability and matrix integrity

After the stress-test, the membranes were exposed through initial approaches as described above (Fig. 3b–c). Two independent investigators, blinded to fixation randomization, visually assessed the membrane fixation integrity as previously described (Table 1) [17]. An additional scoring considering delamination of the membrane was added, especially to assess delamination of the trilayer prototype (Table 1). Delamination was defined as separation of laminae, i.e. membrane layers. This delamination was assessed between the deep porous alar and the additional adhesive layer.

2.6. Comparison

All assessed parameters were compared between the bilayer and the trilayer membrane.

2.7. Statistics

The evaluations of the membrane fixation were compared between the bilayer and the trilayer groups with t-test (two-tailed, heteroscedastic).

Power analysis of the study was performed and sufficient power was considered with a value greater 0.8.

3. Results

Table 2 shows the results of the assessment of fixation integrity. The fixation stability of the trilayer collagen prototype without fibrin glue is lower than of the bilayer membrane with fibrin glue (all $p < 0.05$). No significant differences occurred between medial and lateral talar shoulder location (all $p > 0.05$). No significant differences occurred between the assessment of the two investigators (all $p > 0.05$). The delamination of the trilayer collagen prototype was 1.1 on average (not measured for bilayer by definition).

4. Discussion

This is the first human specimen experimental study for comparison of the fixation stability of the bilayer collagen membrane with fibrin glue (Chondro-Gide, Geistlich Pharma AG, Wollhusen, Switzerland) with the trilayer collagen prototype without fibrin glue in chondral defects at the medial or lateral talar shoulder. Why is this study needed? The fixation of the conventional bilayer membrane with fibrin glue is sufficient and has become the standard for MAST and AMIC [1–11]. However, the use of fibrin glue is expensive, time consuming and may carry a risk of infection [11]. Therefore, implantation without fibrin glue was desired [11]. Unfortunately, the fixation without fibrin glue or sutures was not sufficient [11]. Consequently, the need for a membrane with intrinsic fixation component comparable to fibrin glue would be beneficial [11]. The trilayer collagen prototype seemed to be a reasonable construct for a preliminary testing. Before use in the clinical setting, a human specimen test is useful to assess the fixation stability. In this human specimen experimental test, the fixation stability of trilayer collagen prototype without

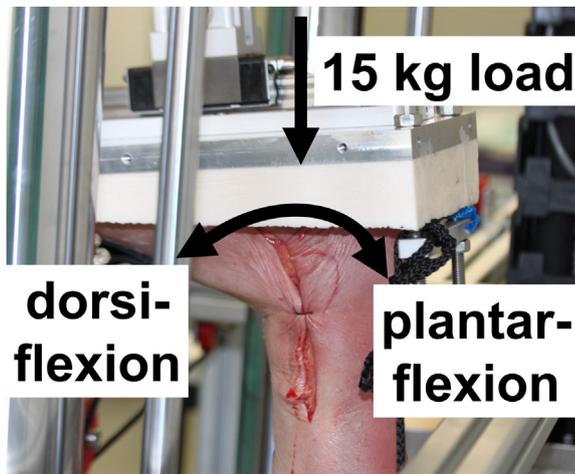


Fig. 4. Test setting. The specimen is mounted upside-down.

Table 1
Scoring system for membrane fixation and delamination.

Peripheral detachment	Score
Detachment <5%	0
Detachment 5%–24%	1
Detachment 25%–49%	2
Detachment 50%–74%	3
Detachment 75%–99%	4
Detachment 100%	5
Area of defect uncovered	
Uncovered <5%	0
Uncovered 5%–24%	1
Uncovered 25%–49%	2
Uncovered 50%–74%	3
Uncovered 75%–99%	4
Uncovered 100%	5
Membrane constitution	
Minimal or no disruption	0
Mild wave formation	1
Moderate wave formation	2
Severe wave formation or prominent folding	3
Delamination	
Delamination <5%	0
Delamination 5%–24%	1
Delamination 25%–49%	2
Delamination 50%–74%	3
Delamination 75%–99%	4
Delamination 100%	5

Minimum (best) score, 0 points; maximum (worst) score 18 points.

Table 2
Evaluation of membrane fixation and delamination after stress test.

	Bilayer	Trilayer	t-test
Peripheral detachment	0.60 ± 1.54	3.40 ± 0.85	<0.001
Area of defect uncovered	0.60 ± 1.54	3.40 ± 0.85	<0.001
Patch constitution	0.06 ± 0.24	0.90 ± 0.31	0.03
Delamination	–	1.1 ± 1.66	

fibrin glue was lower than the fixation stability of bilayer collagen membrane with fibrin glue. Peripheral detachment and area uncovered was 3.4 on average for the trilayer collagen prototype. Both parameters were 0.60 on average for the bilayer membrane plus fibrin glue construct. Patch constitution stability seemed to be high for both (Trilayer 0.90 and bilayer plus fibrin glue 0.06 on average) even though bilayer plus fibrin glue is also more stable than trilayer for this parameter. Relevant delamination of the

trilayer membrane did not occur (Delamination 1.1 ± 1.66 ; not measured for bilayer by definition). We cannot answer the question how much stability is needed in the clinical situation. This leads to different interpretation options of the results: A) bilayer plus fibrin glue is stable enough and trilayer not; B) both are stable enough; C) both are not stable enough. Based on the numerous favourable clinical results reported with the bilayer plus fibrin glue construct, the stability of this construct could be considered to be sufficient [1–11]. So, the option C) both not stable enough could be doubted based on the favourable clinical outcome in different studies [1–11]. The option B) seems to be less realistic than A) based on the high differences in stability of peripheral detachment and area of defect uncovered. However, it is still not known what fixation stability is needed in the clinical situation, and the literature review is also not able to answer this question. Whyte et al. compared different fixation methods (fibrin glue and sutures) of the bilayer membrane in an experimental study with porcine specimen knees [17]. They found that suture increases the stability of fixation to a chondral defect better than fibrin glue alone in the porcine knee after repetitive motion, with respect to patch detachment and chondral defect uncovering [17]. Application of fibrin glue to the base of the defect, or securing the patch with suture, decreased collagen patch deformation [17]. Filardo et al. evaluated stability and integrity of bi-layer and three-layer collagen-hydroxyapatite (C-HA) osteochondral scaffolds in a human cadaveric knee exposed to continuous passive motion (CPM) with and without loading and the role of added fibrin glue to improve the press-fit fixation of C-HA scaffolds [18]. Fibrin glue improved bi-layer or three-layer C-HA scaffold press-fit fixation regardless of lesion location and therefore recommended the use of fibrin glue [18]. Drobic et al. tested four fixation techniques for a fibrinogen and thrombin coated collagen fleece, used as a scaffold in cartilage repair of human cadaveric knees [19]. Fibrin sealant fixation was easy to perform and assured satisfactory scaffold stability [19]. Bone sutures and periosteal cover provided excellent scaffold stability, but the techniques were difficult and caused additional injuries [19]. They recommended regardless of the fixation technique used, avoiding of loading in the initial postoperative period [19]. Bekkers et al. could demonstrate a higher scaffold integrity with fibrin glue compared to transosseous fixation and continuous cartilage sutures [20]. Concerning the location of defects in the knee, Drobic et al. demonstrated that fibrin glue improves the fixation of this collagen-HA scaffold regardless of lesion location, improving implant stability while preserving its integrity [19].

However, these findings are not transferable to the human talus. Especially the joint characteristics with the knee as one of the least congruent joints with thick cartilage and the ankle as one of the most congruent joints with thin cartilage, do not allow to transfer finding whatever kind from one to the other joint [13,17]. Also, in the clinical situation, sutures for membrane fixation in the talus are unusual at all [1–11].

4.1. Limitations

There are numerous potential limitations of this study such as hydration of the membrane with 0.9% NaCl instead of impregnation with BMAC (MAST procedure), questionable compatibility of human specimens with the real clinical situation (for example normal cartilage with iatrogenic defects instead of pathological cartilage in the real clinical situation), questionable bio-compatibility of experimental loading, low specimen number, comparison medial versus lateral talar shoulder with randomization instead of matched-pair specimens.

The hydration of the membrane with 0.9% NaCl solution before implantation in the chondral defect is the standard procedure for

AMIC because the membrane expands by 10% to 15% when hydrated [6]. For MAST, BMAC is used instead of 0.9% NaCl solution [13]. For AMIC + PBC (Peripheral Blood Concentrate), PBC is used instead of BMAC. As BMAC and PBC is also isotonic, use of 0.9% NaCl solution for immersion of the membrane seems to be absolutely appropriate [6,13,14]. The use of human specimens is always a weakness which we cannot invalidate. At least, we used human specimens instead of veterinarian [17]. The questionable biocompatibility of experimental stress testing is also a principal weakness [16]. A physiological loading is not possible regarding the load as such and the ROM [13,16]. Also, the loading in the clinical situation takes weeks, and, in addition, an unknown “healing” process occurs [1–11]. In experimental specimen testing, the loading is condensed to hours and no healing process occurs [16]. Furthermore, the correct ROM is unclear and the ROM in our experimental setting is more than the in the typical clinical postoperative treatment [14]. Then, we did not use match-pair specimens for testing. Instead, we used 10 different individual specimens, and the membranes were randomized to 5 applications at the medial and lateral talar shoulder each. We did not find differences between medial and lateral talar shoulder application which reflects the clinical situation [6,11]. Limited specimen number is also a principal concern for all experimental studies. Ten specimens, i.e. 10 membrane implantations of both membranes still seem to be appropriate. This experimental setting is only appropriate to assess primary stability. Biological processes (adhesion due to blood clotting, . . .) also leading to membrane adhesion cannot be estimated. We are aware of the limitations of the setting but we do believe that our setting is as adequate as others before [17].

In conclusion, the fixation stability of the trilayer collagen prototype without fibrin glue is lower than of the clinically used bilayer membrane with fibrin glue in chondral defects at the medial and lateral talar shoulder in an experimental human specimen test. Clinical use of trilayer collagen prototype without fibrin glue has to be validated by clinical testing to evaluate if the lower stability of fixation is still sufficient.

Conflict of interest

The first author and the co-authors' institution received funding in relation to this study from Geistlich Pharma AG, Wollhusen, Switzerland.

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