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Fibrin glue improves osteochondral scaffold fixation: study on the human cadaveric knee exposed to continuous passive motion

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SUMMARY

Objective: To evaluate 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.
Design: Osteochondral lesions (2.0 × 1.5 cm) were chiseled out on both condyles and trochlea in eight human cadaveric knees. A total of 24 bi-layer (5 mm, four in each condyle) or three-layer C-HA scaffolds (8 mm, eight in the trochlea, four in each condyle) were first press-fit implanted and underwent testing with CPM, 90 cycles, 0°–90°. The second set of 24 scaffolds was implanted in cleaned lesions with the addition of fibrin glue. Two knees with fibrin glue fixation were additionally exposed to 15 kg loading, with 30 cycles of CPM, 0°–30°. Then, the knees were reopened and the scaffolds were evaluated using semi-quantitative Drobnic and modified Bekkers scores.
Results: All but two scaffolds remained in the lesions site throughout CPM. Two implants failed: both were bi-layer osteochondral scaffolds, press-fit implanted at the lateral femoral condyle (LFC). A statistically significant difference was obtained between press-fit and fibrin glue implants with both Drobnic (2.9 ± 0.7 vs 4.3 ± 0.1, $P < 0.0005$) and Bekkers (3.3 ± 1.0 vs 5.0 ± 0.1, $P < 0.0005$) scores. Additional knee loading did not affect fibrin glue scaffold fixation or integrity.
Conclusion: This cadaveric study showed fibrin glue notably improved bi-layer or three-layer C-HA scaffold press-fit fixation regardless of lesion location. It is therefore recommended that fibrin glue be used during surgery to improve early post-operative C-HA scaffold stability and integrity.

Introduction

Cartilage lesions have a high impact on society[1–4]: the greater emphasis on physical activity in all age groups is responsible for the growing rate of these lesions, which are caused by trauma, overuse, or favored by several factors such as misalignment, loss of meniscal tissue, joint instability or laxity[5,6]. Thus, several treatments have been proposed, from conservative to minimally-invasive injective procedures, to surgical strategies to restore a correct biomechanical balance and replace the damaged articular surface[7–9]. An increasing awareness of the role of the subchondral bone in the[10] has led to the recent development of osteochondral scaffolds[11]. Among these, one was designed for large lesions: a nano-structured biomimetic collagen/hydroxyapatite (C-HA) scaffold (MaioRegen: Fin-Ceramica S.p.A., Faenza, Italy). Animal studies showed good and similar results when implanting the scaffold loaded with autologous chondrocytes or the scaffold alone[12–14]. Therefore, C-HA scaffold was introduced into clinical practice as a non-cellular device. Positive clinical results have been shown by isolated reports and short-term case series and a recent study seem to confirm the good outcome also at mid-term[15–17]. These promising findings have been strengthened by results in more challenging lesions, such as large complex defects or even in patients affected by unicompartmental osteoarthritis[18–20]. However, there are some drawbacks: not all patients benefit from this procedure, post-operative adverse events have been reported, and imaging analysis shows controversial findings[17]. An Magnetic Resonance Imaging (MRI) study by Kon *et al.* that evaluated the early stability of implanted scaffolds[21] documented partial detachment in 13%, a complete filling in only 67%, and a complete integration of the

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grafted cartilage in only 53% of the lesions after 6 months. These problems may be attributable either to the scaffold itself, or to the implantation technique based on a press-fit fixation. Optimal stability of the implanted scaffold in the first post-operative phase is of the utmost importance particularly for a scaffold also designed for the treatment of large and complex defects.

The aim of the study was therefore to evaluate the early post-implantation scaffold stability, integrity, and fixation strength in a human cadaver knee exposed to continuous passive motion (CPM) and to upgrade the implantation surgical technique. The hypothesis was that the addition of fibrin glue might provide a mechanical benefit and should be applied to improve the stability of the implanted scaffold.

Methods

Permission for experimental work was granted by the National Ethical Commission. The study set-up focused on the evaluation of osteochondral scaffold stability, with or without the use of fibrin glue, during the operative procedure and during mobilization in the initial post-operative period in a previously developed human cadaveric knee model[22].

Osteochondral scaffold

The nano-structured biomimetic scaffold has a porous 3-D three-layer composite structure. The cartilaginous layer, consisting of type I collagen, has a smooth surface to favor joint flow. The intermediate tide-mark-like layer consists of type I collagen (60%) and hydroxyapatite (HA) (40%), whereas the subchondral bone layer consists of a mineralized blend of type I collagen (30%) and HA (70%). Each layer is separately synthesized from an atelocollagen aqueous solution (1% w/w) in acetic acid, isolated from equine tendon. The final construct is obtained by physically combining the layers on top of a Mylar sheet and finally freeze-drying and gamma-sterilizing it at 25 KGray. Besides the three-layer scaffold, already used in the clinical practice, a modified thinner bi-layer scaffold, developed to increase the physical properties and to target shallower cartilage lesions, was also tested. This scaffold consists of an upper cartilaginous layer and a lower subchondral layer. For the synthesis of the cartilaginous layer 200 g of 1 wt% type I collagen in acid aqueous suspension (pH = 3.5) were diluted with 200 mL of milli-Q water and were then precipitated by dropping NaOH solution 0.1 M up to the isoelectric point (pH 5.5). The fibers were subsequently washed three times with 300 mL of milli-Q water. For the synthesis of the subchondral layer a solution prepared with 3216 g of H3PO4 85%v/v and 700 mL of milli-Q water was mixed with 300 g of 1 wt% type I collagen gel and dropped into 1100 mL of basic suspension containing 1.554 g of Ca(OH)2 95 wt%, 0.2024 g of MgCl2·6H2O and 55 mL of SBF to yield a composite Mg-HA/Collagen material in the theoretical ratio of 70/30 wt% and Mg/Ca mol = 5% in the crystal lattice. The precipitated fibers were matured for 1 h and subsequently washed three times with 400 mL of milli-Q water. With the aim of stabilizing the scaffold and improving its resistance to physiologic enzymatic attachment, each layer was chemically cross-linked through a 48-h treatment at 4 °C in 0.05 wt % aqueous solution of 1,4-butanediol diglycidyl ether bis-epoxy (BDDGE). Both scaffolds were precisely cut out with a scalpel under caliper control to obtain homogeneous dimensions of 2 cm in length and 1.5 cm in width.

Osteochondral lesion preparation

Eight fresh-frozen human cadaveric lower limbs, exarticulated at the hip joint, were used in the study. They were removed from the freezing storage 12 h prior experiments, and they were slowly thawed and wormed up at ambient temperature, and kept at room temperature (20 °C) throughout the experiments for approximately 24 h. None of the limbs had detectable misalignment; ligaments, menisci and cartilage in the knees were intact either on testing or visual examination. The articular surfaces were exposed through a medial parapatellar arthrotomy. The width of the central parts of the articulating femoral surfaces was the following: medial femoral condyle (MFC) from 24 to 33 mm, lateral femoral condyle (LFC) from 29 to 35 mm, and the width of the articular surface on the central part of the trochlea from 41 to 55 mm. Rectangular osteochondral lesions were prepared with an osteotome under caliper control to obtain precise defect areas of 2 times 1.5 cm (Fig. 1). More in detail, the osteotome was used by performing a superficial delimitation of the lesion first, and subsequently a series of parallel incisions alternated by perpendicular ones to delicately remove fragments of the degenerated tissue leaving intact and perpendicular defect shoulders. The process was repeated to obtain vertical margins and gradually deepen the lesion area according to the

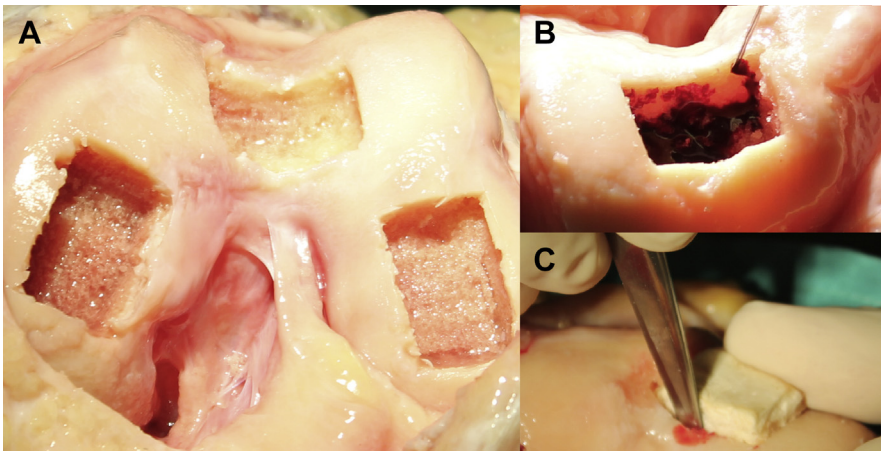

Fig. 1. The osteochondral scaffolds are implanted in condyle and trochlea lesions of 2 × 1.5 cm (A), filled with blood to reproduce the initial phases of blood impregnation and coagulation in the clinical practice (B). Tips and tricks (C): if the press-fit insertion is cumbersome, to avoid deformation of the scaffold caused by friction of the lower scaffold layers with the subchondral bone, with consequent layer disruption bringing HA to the surface level, it can be helpful to accompany the insertion of the lower scaffold layers with a thin metal instrument, "shoehorn" style.

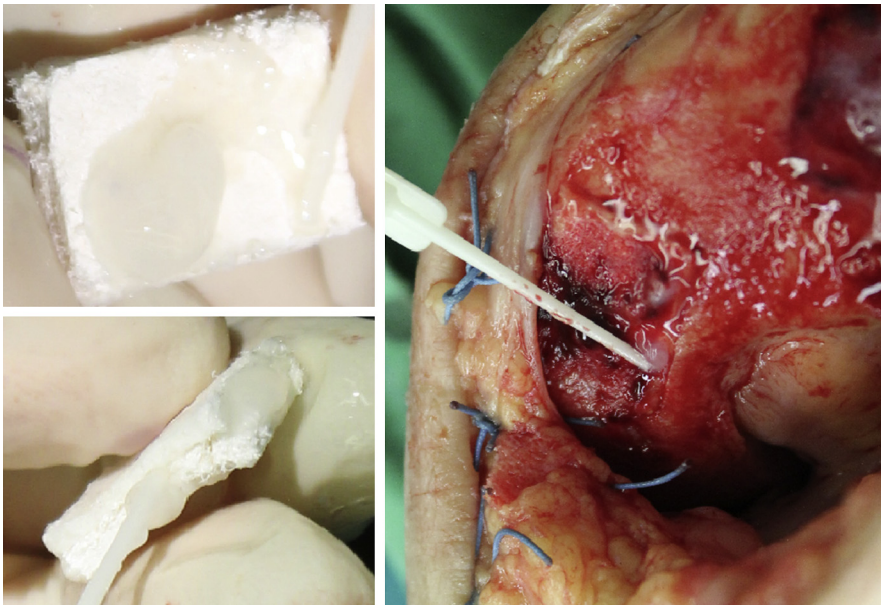

Fig. 2. Fibrin glue is applied to the bottom part of the scaffold (upper left) and its margins (lower left); then after implantation it is applied to the host–scaffold interface and implant surface (right).

requirements of the scaffold to be implanted. The lesion depth was defined according to the manufacturer's recommendations: 8 mm for three-layer C-HA scaffold and 5 mm for the bi-layer one. The distributions were as follows: all trochlear lesions hosted only thick three-layer scaffolds, whereas 4 MFC and 4 LFC were prepared for the three-layer scaffolds, and 4 MFC and 4 LFC lesions were prepared for the bi-layered ones. Just before each scaffold implantation the lesions were filled with fresh blood (2–3 cc for each lesion were used) from a healthy donor to allow scaffolds to be soaked in blood and reproduce the first phases of blood impregnation and coagulation in the clinical practice (Fig. 1).

Fixation of the collagen scaffold

A total of 24 scaffolds, eight on the trochlea and eight on each condyle, were implanted first; eight bi-layer and eight three-layer scaffolds were equally distributed between both femoral condyles, whereas all trochlear lesions were treated with the three-layer scaffold. All scaffolds were press-fit implanted and underwent the CPM protocol. After evaluation, the first set of scaffolds was discarded and the lesions were cleaned. The second set of 24 scaffolds was implanted with fibrin glue (2 cc were sufficient for the fixation of each scaffold) before CPM. Fibrin glue (Tisseel, Baxter AG, Vienna, Austria) was first applied to the bottom, bone part, of the scaffold and to the side walls. When the scaffold was implanted it was impregnated with blood on the surface, and then care was taken to apply fibrin glue to the host–scaffold interface, and finally a layer of fibrin glue was applied to cover the scaffold surface (Fig. 2).

Mobilization protocol

After fixation of all three scaffolds, 5 ml of lactated Ringer's solution was added intra-articularly to moisten the joint surfaces. The arthrotomy wound was closed in layers with interrupted sutures. The whole inferior extremity was then strapped to the CPM device (Kinetec, Ormed GmbH & Co., Freiburg, Germany). One motion cycle, extension (0°) to flexion (90°) and to extension (0°), lasted 90 s (Fig. 3)[22,23]. After 60 cycles the arthrotomy wound was reopened to evaluate the scaffold's appearance. This sequence was

Fig. 3. The lower limb is strapped to a CPM device to perform 60 motion cycles of 90 s (0°–90°–0°) (left). To simulate partial loading elastic straps are stretched between a Steinman pin in the distal femur and the foot, and a force transducer is incorporated to control the 15 kg force applied between the two joints (right).

repeated twice: with press-fit scaffolds and with the scaffolds fixed with fibrin glue. The scaffolds in the last two tested extremities (both with three-layer scaffolds at the trochlea level, whereas the bi-layer and three-layer scaffolds were alternated in the MFC and LFC in the two knees) fixed with fibrin glue were additionally exposed to 30 cycles of CPM loading to simulate the partial weight bearing performed postoperatively. For this purpose, elastic straps were stretched between a Steinman pin in the distal femur and the foot. A force transducer was incorporated into the elastic system (Fig. 3). Nominal loads of 15 kg were applied. The motion cycles under loading were limited to: extension (0°) flexion (30°) and extension (0°). The force deviations between flexion and extension were detected on the transducer, but excursions from the nominal load did not exceed 20% in both directions. After completion of CPM loading the knees were bent to 90° and reopened for the final evaluation.

Evaluation

The scaffolds were macroscopically evaluated by three surgeons independently. A consensus of all three evaluators was reached as the end result. The evaluation was performed with two semi-quantitative scores. The Drobnic score[22] combines three scales: area coverage (range from 0 (>50% of area uncovered) to 5 (unchanged)), integrity of the scaffold (range from 0 (>50% of scaffold lost) to 5 (unchanged)), and manually tested endpoint fixation strength (range from 0 (total self-detachment) to 5 (not detachable)). The Bekkers score[23] was modified by removing the last scale dedicated to the endpoint fixation strength measured quantitatively but a pulley-block system was not used, and consisted of: outline attachment (range from 0 (100% circumference lost contact) to 5 (unchanged)), area coverage (range from 0 (0%) to 5 (unchanged)), and scaffold integrity (range from 0 (general scaffold disorganization/cracks jeopardizing the fixation) to 5 (unchanged)).

Strength testing was performed after the completion of 60 CPM cycles, except in the last two extremities in which the fixation strength of the fibrin glue implanted scaffolds was tested after the completed loading protocol. The values obtained by the average of the different scales for each score were used for the study purpose.

Statistical analysis

The Kolmogorov–Smirnov test was performed to test normality of continuous variables. The logarithm transformation was used to make the score distributions Normal and Homoscedastic. The Repeated Measures General Linear Model (GLM) with the knee as the random effect was performed to assess the differences at different follow-up times. The GLM with the knee as the random effect and the scaffold and site as the fixed effect was performed to test the influence on the scores of the scaffold in the different sites. The Mann–Whitney or the Kruskal–Wallis non-parametric test evaluated by the Exact method for small samples were performed to assess the between or among group differences. For all tests $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS v.19.0 (IBM Corp., Armonk, NY, USA).

Results

A statistically significant difference was obtained between the press-fit and fibrin glue implants in both Drobnic and Bekkers scores (Fig. 4). In particular, the evaluation with the Drobnic score showed: the press-fit implants after CPM presented a value of 2.9 (CI 2.6–3.2), whereas the fibrin glue implants reached a value of 4.3 (CI 4.3–4.3) ($P < 0.0005$). The Bekkers score was 3.3 (CI 2.8–3.7) for press-fit implants and 5.0 (CI 4.9–5.0) for fibrin glue implants ($P < 0.0005$). A further analysis was performed to evaluate site-related differences in the Drobnic and Bekkers scores obtained both by press-fit and fibrin glue implants: no statistically significant difference was found (press-fit Drobnic score $P = 0.2$; fibrin glue Drobnic score $P = 1.0$; fibrin glue Bekkers score $P = 1.0$), and only a tendency among press-fit implants was seen with better Bekkers scores in the MFC ($P = 0.076$) (Fig. 5). Furthermore, no significant score differences were found comparing the results obtained for the femoral condyles treated either with the three-layer or the bi-layer scaffolds (press-fit Drobnic score $P = 0.6$; fibrin glue Drobnic score $P = 1.0$; press-fit Bekkers score $P = 0.8$; fibrin glue Bekkers score $P = 1.0$). The only observed difference was found by analyzing the effect of site and scaffold together: GLM showed that whereas in the MFC the two scaffolds had similar results, significantly lower scores were documented for LFC treated with the bi-layer scaffold,

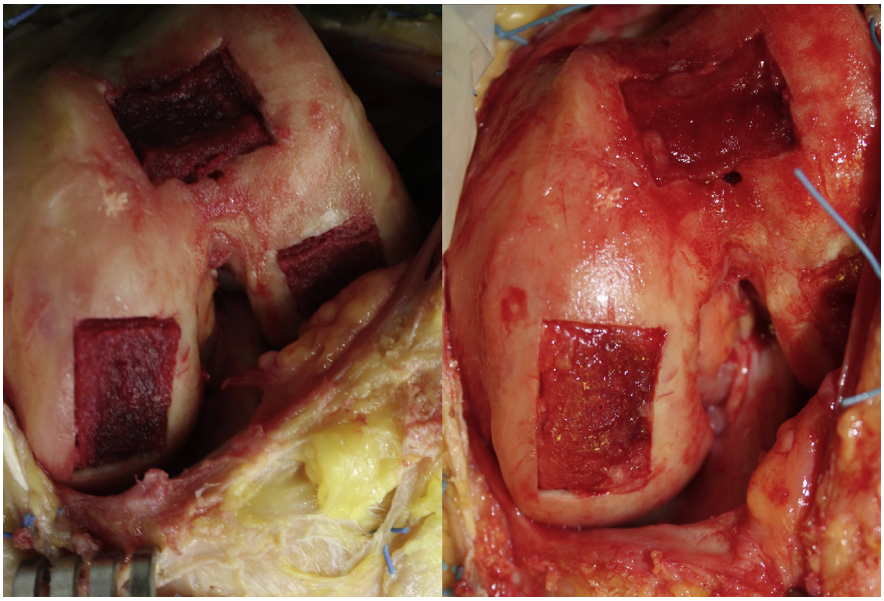

Fig. 4. Implant appearance after CPM of press-fit implanted only scaffolds (left) or press-fit plus fibrin glue (right).

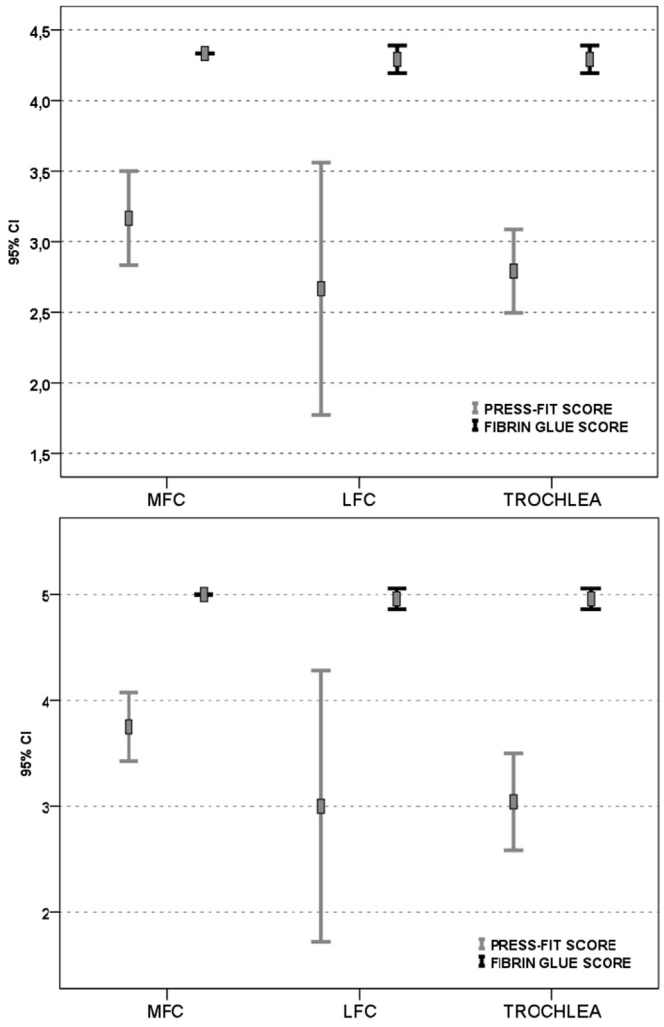

Fig. 5. No statistically significant difference in both Drobnic (upper part) and Bekkers (lower part) scores (mean and 95% confidence interval of the mean are reported) was found among implants of the trochlea, MFC, and LFC; only a tendency among press-fit implants was seen with better Bekkers scores in the MFC ($P = 0.076$).

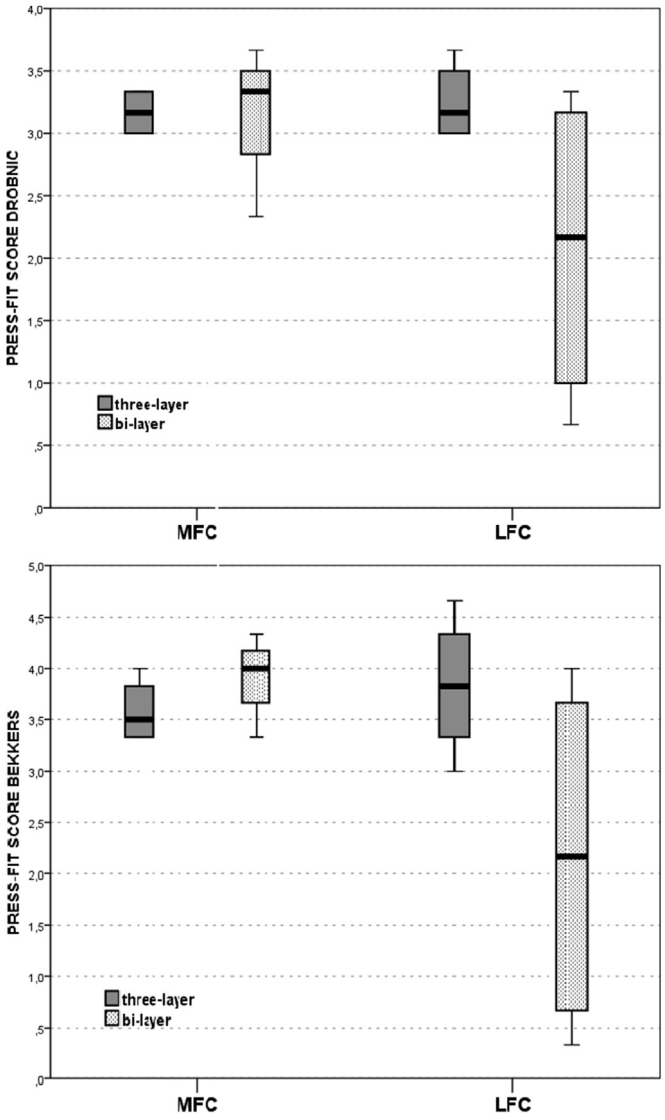

Fig. 6. Similar results were obtained with the three-layer and the bi-layer scaffolds (results are reported in box and whisker plots: the central bold line representing the median, the box limits representing the quartiles, and the whiskers representing the minimum and maximum values). The only difference was found by analyzing the effect of site and scaffold together with GLM: whereas in the MFC the two scaffolds had similar results, lower scores were documented for LFC treated with the bi-layer scaffold, both with the Drobnic (upper right) and Bekkers (lower right) scores (both $P = 0.04$).

both with the Drobnic ($P = 0.04$) and Bekkers ($P = 0.04$) scores (Fig. 6). Loading in the two knees of the fibrin glue group and subsequent further mobilization did not affect the stability or produce any detectable changes in the implanted scaffolds. Two implants clearly failed: both cases were bi-phasic osteochondral scaffolds, press-fit implanted at the LFC, which presented a marked disruption with delamination and layer dislodgement (Fig. 7). Some final considerations are not quantifiable and are reported as qualitative observations. The evaluation of the press-fit implanted scaffolds showed that the scaffold swelling due to its interaction with biologic fluids was not homogeneous, resulting in loss of press-fit at the cartilage level and exposure of the lower C-HA structure directly at the intra-articular space. This problem may appear already during the implantation and is worsened by CPM, whereas it did not affect the fibrin glue implanted scaffolds, not even after loading. The scaffold pattern without fibrin glue can be summarized in two alterations that might be hypothesized as failure mechanisms (Fig. 8). The first one is the deformation, bending and dislodgment of the entire scaffold; the second one is the delamination with destruction of the cartilage layer. This was never observed when using fibrin glue, which stabilized the scaffold both by increasing its internal layer cohesiveness and the integration in the lesion area. The application of high amounts of fibrin glue did not jeopardize the scaffold. In fact, mobilization caused the removal of the fibrin glue layer in excess without affecting the integrity and stability of the upper cartilage scaffold layer. Finally, the bi-layer scaffold presented good handling properties and less deformation after interaction with biologic fluids, but this did not change the results in terms of implant stability and integrity after CPM. Thus, the different locations and the different scaffolds confirmed that fibrin glue makes a significant mechanical difference in all implant conditions.

Discussion

This *ex-vivo* study addressed press-fit and press-fit plus fibrin glue fixation of two-layer and three-layer osteochondral scaffolds in cadaver knees exposed to CPM with and without loading, to

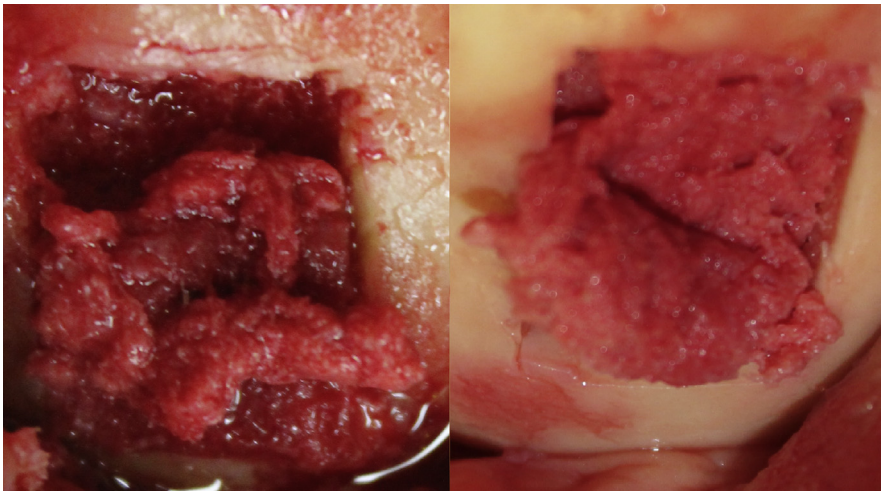

Fig. 7. Two bi-phasic osteochondral press-fit implanted scaffolds failed at the LFC: a marked disruption with delamination and layer dislodgement can be observed.

simulate the early post-operative phase. Fibrin glue significantly improved the stability and integrity of the scaffolds irrespective of lesion location or scaffold type. To the authors' knowledge, this is the first study to specifically analyze and optimize the implantation technique of this C-HA scaffold already applied in the clinical practice. Literature analysis shows that clinical studies on this C-HA scaffold reported an overall good outcome but also some controversial aspects[15–21] that might be, at least in part, explained by this experiment. An insufficient fixation coupled with an aggressive rehabilitation might lead to scaffold damage, delamination, synovitis induced by HA release, and even implant failure. To avoid these problems and further improve the initial positive clinical results, fibrin glue is an option to increase the press-fit stability of this scaffold.

Scaffold fixation can be obtained with several methods: press-fit only or intrinsic adhesive scaffold properties, fibrin glue, suture, pin fixation[22–24]. Whereas some authors suggest a low mechanical stabilization of fibrin glue, inferior with respect to other more invasive options[25] (which on the other hand present problems such as more cumbersome procedures and risk of further iatrogenic joint damage[23]), others show that fibrin glue still offers an additional fixation and may significantly improve the early fixation rate[22,26,27]. To evaluate the contribution of fibrin glue to fixation a human cadaver model was used as introduced by Drobnic *et al.*[22] and Bekkers *et al.*[23], who compared the stability and fixation of thinner chondral scaffolds in a condition comparable to the post-operative phase. This *ex-vivo* study allowed a precise comparison of the two fixation techniques directly in the same setting, thus demonstrating the additional value of fibrin glue fixation. The key role of fibrin glue in enhancing mechanical fixation and therefore scaffold stability and integrity was clearly shown in all experimental conditions. Statistically better scores were obtained in all locations, MFC, LFC, and trochlea, and with both the three-layer scaffold and the thinner bi-layer version. The bi-layer version has been developed aiming to treat shallower defects, where the subchondral bone is still involved, but that require a less deep lesion preparation to remove the degenerated layer and expose vital subchondral bone. Interestingly the two incontrovertible failures both occurred in LFCs treated with the bi-layer scaffold, which might be explained by unfavorable conditions due to a combination of defect shoulder depth and curvature of the articular surface. However, regardless of the failure characteristics, the contribution of fibrin glue to scaffold stability was shown to be critical in all conditions. After applying fibrin glue the implants improved both in terms of intrinsic layer cohesiveness and fixation strength at the articular surface. This finding has two major consequences, one is understanding the behavior of the scaffold described in the current literature, and the other in suggesting a better implant technique and post-operative management. Understanding press-fit implanted scaffold behavior mainly regards the possible mechanisms of failure. It is of particular interest to notice that two out of 16 condyles failed, making a 12.5%, rate which is similar to the 13.3% imaging findings of partial detachment reported in a previous MRI early stability evaluation study[21]. Two failure mechanisms can be hypothesized: the entire

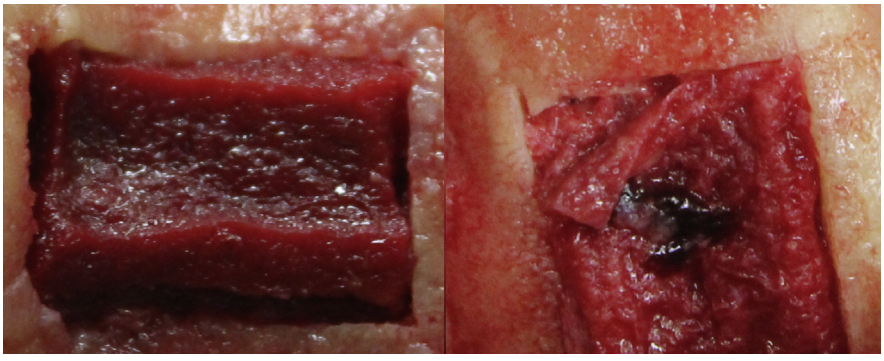

Fig. 8. Two possible failure mechanisms of press-fit implanted scaffolds without fibrin glue: deformation, bending, and dislodgment of the entire scaffold (left) or delamination with destruction of the cartilage layer (right).

osteochondral scaffold can be deformed and dislodged, thus leading to a loose body, or the superficial layer can be altered with delamination and disruption of the cartilage-like surface. This second mechanism might be more difficult to detect, since the damage or loss of the upper layer might be much more difficult to observe by MRI with respect to a completely empty defect after implant detachment. The characteristics that might lead to the two failure patterns cannot be precisely demonstrated, but it is likely that a role is played by the articular site (with peculiar surface curvature and mechanical stresses), since it was more likely to find the former at the trochlea level, whereas delamination was observed mainly at the condyle level. Regardless of the modality, these mechanisms have the exposure of the lower layers and therefore the release of HA in the joint environment in common, which can still cause, if not a failure, swelling and temperature[28,29], which are adverse events responsible for a slower clinical improvement. This aspect also leads to repercussions of the study results on surgical and postsurgical patient management. In fact, whereas the press-fit implantation might cause a lack of stability with slow scaffold integration and post-operative adverse events, which have been shown to affect the clinical outcome up to 2 years of follow-up[16], a stable implant allows a safer and faster rehabilitation. Fibrin glue fixation would allow an early mobilization that both would remove the fibrin glue layer in excess (preventing adhesion formation) and would allow a safer progressive load. In fact, in the treatment of musculoskeletal injuries one of the most important developments has been the understanding that controlled early resumption of activity can promote restoration of function[30]. Joint movement and weight bearing are necessary for tissue homeostasis, by ensuring nutrition and maintaining biomechanical properties of normal cartilage. *In vitro* studies have shown that mechanical forces affect cell biosynthesis and gene expression[31,32]. The repair tissue after scaffold implantation is also exposed to a remodeling process and thus susceptible to mechanical stimuli. Moreover, a fast rehabilitation might not just shorten the implant maturation time, but might also reduce the initial complications due to immobilization and inactivity and even significantly improve the final long-term clinical outcome[33]. Of course, the implant is most vulnerable during the initial stage and should be protected by avoiding peak compressive and shearing forces[34]. However, the results of the present study show that fibrin glue stabilizes the implant, thus allowing a safe early mobilization, and even slight joint loading resembling the toe-touch weight bearing does not seem to jeopardize the implant. It would therefore seem a reasonable assumption that the increased mechanical stability with the addition of fibrin glue might allow safe management and probably speed up the immediate post-operative period, and might even optimize the results of surgery. The following study limitations need to be acknowledged: short post-implantation testing time, *ex-vivo* nature of the study, which lacks information on the intra-articular biology on scaffold stability, and limited sample size. However, the 60-cycle testing time was selected according to a previous study showing that the effect of mobilization on scaffold stability and integrity, as well as failures, were detected at this time point, whereas further mobilization cycles did not offer any further information. The toe-touch weight bearing was simulated only in two knees and only when the scaffold was fixed with fibrin glue. There was no reason to stress the press-fit implant further, since the CPM mobilization already clearly outlined the criticalities of this fixation. Lesions were not large, but they are similar to those reported for large cartilage treatment databases[24,35]. Moreover, smaller lesions should in theory favor the shoulder protection of the graft: therefore, the highly significant results should make the positive effect of fibrin glue plausible also for bigger lesions. The main limitation of this study is the pure mechanical investigation of the fixation modality. In fact, whereas there is incontrovertible evidence on the mechanical advantage of fibrin glue both in terms of scaffold stability and protection, we do not know the biological interaction in the living joint environment. In spite of the *ex-vivo* model, fresh blood from the by-standing donor was applied to the lesion to simulate natural fixation and cohesive element acting on the scaffold *in vivo* immediately after implantation. Another aspect that cannot be explored by this model is that fibrin glue might obstruct the migration of mesenchymal stem cells from the surrounding subchondral bone, as well as the flow of nutrients, and therefore might affect the initial integration and maturation phases. However, this is an unlikely situation, since fibrin glue has been used in cartilage treatments for decades and recently has even been suggested as a suitable scaffold for chondrocyte transplantation[11,36–38]. In this light, fibrin glue might favor implant integration not only mechanically, but also biologically, by representing a scaffold itself to host cells and promote tissue formation at the host–scaffold interface. Whereas the biological effect of fibrin glue has to be further explored through preclinical *in vivo* studies, it has to be underlined that scaffold stability is a prerequisite for the success of an implant. Thus, until other studies increase the knowledge on the initial phases of implant integration and maturation and give further indications on the most appropriate surgical technique, the results of this study strongly support the use of fibrin glue for the implantation of these bi or three-layer C-HA osteochondral scaffolds.

Conclusion

An evaluation of press-fit implanted scaffolds showed that scaffold swelling due to interaction with biologic fluids is not homogeneous, resulting in the loss of press-fit at the cartilage level and exposure of the lower C-HA structure directly at the intra-articular space, other than delamination and dislodgment. These problems were never observed when using fibrin glue, which stabilized the scaffold both by increasing its internal layer cohesiveness and integrity in the lesions area. This cadaveric study showed that fibrin glue notably improved the press-fit fixation of bi-layer or three-layer C-HA scaffolds regardless of lesion location. It is therefore recommended that fibrin glue be used during surgery to improve early post-operative C-HA scaffold stability and integrity.

Contributions

G. Filardo: study design, study execution, manuscript writing.
M. Drobnic: study design, study execution, manuscript editing.
F. Perdisa: study design, study execution.
E. Kon: study design, manuscript editing.
M. Hribernik: consultant, manuscript editing.
M. Marcacci: senior consultant, manuscript editing.

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Competing interests

M. Marcacci receives royalties and research institutional support from Fin-Ceramica Faenza SpA, Italy.

E. Kon is consultant for CartiHeal (2009) Ltd, Israel and has stocks of CartiHeal (2009) Ltd, Israel, and she received fees for paid presentation from Fin-Ceramica, Italy, and Fidia, Italy.

M. Drobnic is consultant for CartiHeal (2009) Ltd, Israel.

G. Filardo, F. Perdisa, M. Hribernik have nothing to disclose.

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