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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

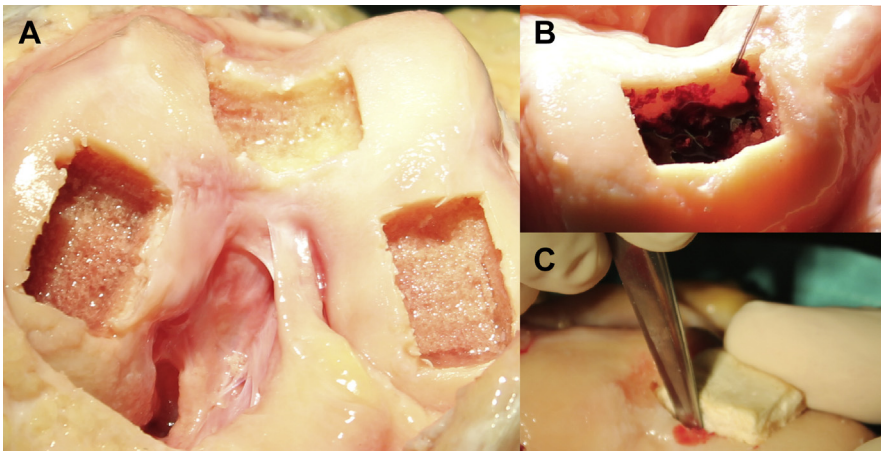

Fig. 1. The osteochondral scaffolds are implanted in condyle and trochlea lesions of 2 × 1.5 cm (A), filled with blood to reproduce the initial phases of blood impregnation and coagulation in the clinical practice (B). Tips and tricks (C): if the press-fit insertion is cumbersome, to avoid deformation 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.