Differences in kinematics of single leg squatting between anterior cruciate ligament-injured patients and healthy controls

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Abstract Seventy to eighty percent of all anterior cruciate ligament (ACL) injuries are due to non-contact injury mechanisms. It has been reported that the majority of injuries due to single leg landing come from valgus positioning of the lower leg. Preventing valgus positioning during single leg landing is expected to help reduce the number of ACL injuries. We found that many ACL-deficient patients cannot perform stable single leg squatting. Therefore, we performed 3D motion analysis of the single-legged half squat for ACL-injured patients to evaluate its significance as a risk factor for ACL injuries. We evaluated the relative angles between the body, thigh, and lower leg using an electromagnetic device during single leg half squatting performed by 63 ACL-injured patients (32 males, 31 females) the day before ACL reconstruction and by 26 healthy control subjects with no knee problems. The uninjured leg of ACL-injured male subjects demonstrated significantly less external knee rotation than that of the dominant leg of the male control. The uninjured leg of ACL-injured female subjects demonstrated significantly more external hip rotation and knee flexion and less hip flexion than that of the dominant leg of the female control. Comparing injured and uninjured legs, the injured leg of male subjects demonstrated significantly less external knee and hip rotation, less knee flexion, and more knee varus than that of the uninjured leg of male subjects. The injured leg of female subjects demonstrated more knee varus than that of the uninjured leg of female subjects. Regarding gender differences, female subjects demonstrated significantly more external hip rotation and knee valgus than male subjects did in both the injured and uninjured legs ($P < 0.05$). The current kinematic study exhibited biomechanical characteristics of female ACL-injured subjects compared with that of control groups. Kinematic correction during single leg half squat would reduce ACL reinjury in female ACL-injured subjects.

Keywords Anterior cruciate ligament (ACL) ·
Single leg squat · Kinematic analysis · Injury prevention

Introduction

Several reports have examined the epidemiology of anterior cruciate ligament (ACL) injury. Seventy to eighty percent of all ACL injuries are due to non-contact injury mechanisms [1–3]. The ACL-injury rate of female athletes is 2–8 times higher than that of male athletes [4–6]. The ACL non-contact injury rate of female athletes is reportedly 2–4 times higher than that of male athletes in basketball and soccer [7]. The majority of ACL non-contact injuries occur in the valgus position of the lower leg during single leg landing and cutting maneuvers in sports activities [8, 9]. Therefore, preventing valgus positioning of the lower leg during single leg landing or cutting maneuvers is expected to reduce the number of ACL non-contact injuries. A number of biomechanical research studies examining jump landing have investigated the injury mechanism of the ACL. They have mainly reported on lower extremity

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kinematics during bilateral or unilateral jump landing or evaluated the effect of neuromuscular training [10–22].

A number of non-contact ACL injuries occur when one leg is overloaded during landing, cutting, and pivoting. If a single leg standing position could be maintained without collapsing during knee bending with weight-bearing during such decelerating maneuvers, ACL injuries could possibly be prevented. Therefore, we asked ACL-injured patients to perform a single leg squat and evaluated their performance pre- and postoperatively at an outpatient clinic.

We assumed that proper control during the single leg half squat depicts ability for safe landing for the ACL. This task is considered to be a simple and safe clinical examination to correct unstable valgus positioning of the lower leg in ACL-injured patients. However, based on clinical observations of ACL-injured patients, a number of female ACL-injured patients cannot perform a stable single leg half squat.

Therefore, we expected some kinematic differences to exist between female ACL-injured patients and healthy controls performing a single leg half squat. The ACL reinjury rate is speculated to decrease if a higher number of ACL-injured patients can perform a stable and correct single leg half squat. Therefore, this method could be a valuable screening tool for identifying predisposition for ACL rupture in healthy individuals. Even more, it could indicate the magnitude of functional disability in ACL-injured patients.

The main purpose of this study is to compare the kinematics of uninjured legs in ACL-injured patients and healthy controls during single leg squatting. Clarification of the characteristics of uninjured leg in ACL-injured patients might reveal a predisposing factor of ACL injury. To identify further details of the characteristics of ACL-injured patients, we evaluated the differences between ACL-injured and -uninjured knee in each male and female, and also gender differences in both injured and uninjured knee.

The primary hypothesis is that some characteristic differences occur between kinematics of ACL-injured patients and healthy controls performing a single leg squat for each gender. The secondary hypothesis is that some characteristic differences exist between the kinematics of ACL-injured knee and the uninjured knee in both males and females. The third hypothesis is that some characteristic differences exist between the kinematics of male and female ACL-injured patients in both injured and uninjured knee.

Materials and methods

A total of 63 ACL-injured patients (32 males, 31 females) participated in this study before ACL reconstruction. The

mean age of the male patients was 26.4 years old (16–51) and 25.5 years old (14–47) for female patients. The period after ACL injury was 3.5 ± 1.8 months for male patients and 3.0 ± 1.7 months for female patients. Various kinds and levels of sports and injury mechanisms were included in this study; however, most patients participated in just recreational level sports. They had not undergone a special rehabilitation program before ACL reconstruction; however, they had sufficient conservative treatment to achieve daily living with no difficulty and knee motion with no remarkable limitation before surgery.

To be included in this study, subjects had to have no history of other lower extremity injuries. Examples of injuries that excluded participation were grade II or higher MCL injury, meniscal locking and tears with severe pain, bilateral ACL injury, and current symptoms of pain induced due to other reasons (visible joint effusions, patella instability, or lower extremity fractures). Subjects with a history of chronic conditions such as tendinitis or bursitis causing no pain at the time of evaluation were included in this study. As a control group, 26 healthy athletes were recruited to participate in this study. The demographic data for control subjects came from 14 male recreational level athletes (basketball $N = 4$, volleyball $N = 2$, skiing $N = 2$, and others) and 12 female competitive level athletes of volleyball. The mean age of the male control subjects was 26.2 years old (22–35) and 23.2 years old (19–33) for female control subjects. The subjects also had no history of any lower extremity injury, including ACL injury. Before starting this study, subjects were informed of the purpose and possible risk of the study and so forth and signed an informed consent form of the university.

The day before ACL reconstruction, each ACL-injured subject was asked to perform a single leg half squat as one of the preoperative clinical examinations. Subjects were instructed and given one opportunity to practice the single leg half squat maneuver. They were instructed to cross their arms over their chest and to perform a half squat while keeping proper balance, with the duration of the squat being 10 s or less. The depth of the squat was defined as the position for maintaining balance well during the 10 s maneuver (Fig. 1). Subjects performed two single leg half squats with both the injured and uninjured legs, while subjects of the control group performed squats with the dominant leg. All other data of the joint angles were extracted from data at maximum knee flexion during the single leg half squat. We measured these joint angles for two occurrences of single leg half squatting.

Instrumentation

3D motion analysis was performed using an electromagnetic device (FASTRACK; POLHEMUS, Colchester, VT),
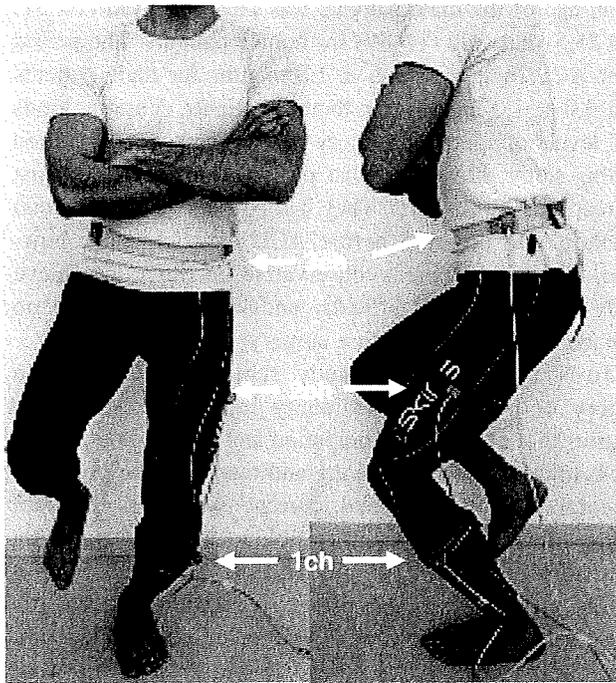
Fig. 1 Subjects were instructed to practice a single leg half squat maneuver. They were then instructed to cross their arms over their chest and to perform a half squat while maintaining proper balance. *Arrows* show receivers (1ch, 2ch, 3ch) attached to the anterior superior iliac spine, 10 cm above the patella on the lateralis of the thigh, and 15 cm below the patella on the lateral side of the lower leg

which can measure six degrees of freedom in a Cartesian coordinate system. This system's software can measure the 3D position of the receiver relative to a global coordinate system projected by the magnetic transmitter. The experiment was organized, so that there was no interference from magnetic materials. The accuracy of the cutaneous measurements was $1.6° \pm 1.3°$, the repeatability of the cutaneous measurements was $0.8° \pm 0.4°$, and that of the transosseous measurements was $1.0° \pm 0.5°$ [23]. This accurate and reproducible device provides a simple and noninvasive measurement. Therefore, it is well suited for biomechanical applications such as joint movement analysis.

Three of the receivers (1ch, 2ch, 3ch) were attached by a circumferential velcro strap placed on the anterior superior iliac spine, 10 cm above the patella on the lateral side of the thigh, and 15 cm below the patella on the lateral side of the lower leg (Fig. 1). This electromagnetic device tracks the relative position of the receivers attached to the anterior superior iliac spine, thigh, and lower leg. This system provides specified accuracy when receivers are located within 76 cm of the transmitter. The static accuracy of this system is 0.08 cm root mean square for the X, Y, and Z values of the receiver's position, and 0.15° root mean square for the receiver orientation.

Statistical analysis

The null hypothesis for this study was that there would be no differences in any kinematic data between the ACL-injured patients and the control group, nor between an ACL patient's injured and uninjured knee, nor a difference between the genders in the ACL-injured patients. Statistical means and standard deviations (SDs) for all measured variables were calculated for each subject group. Kinematic data were analyzed by StatView 5.0 (SAS, Cary, North Carolina) using one-way analysis of variance tests and Fisher's PLSD as a post hoc test. The level of significance was set at $P < 0.05$.

Results

Comparing the ACL-injured subjects to the healthy controls, the uninjured leg of male ACL-injured subjects demonstrated significantly less external knee rotation than the dominant leg of the male controls. The uninjured leg of female ACL-injured subjects demonstrated significantly more external hip rotation and knee flexion and less hip flexion than the dominant leg of the female controls (Table 1; Fig. 2).

Comparing the injured leg with the uninjured leg in ACL-injured subjects, the injured leg of male demonstrated significantly less knee and hip external rotation, less knee flexion, and more knee varus than the uninjured leg. The injured leg of female subjects demonstrated more knee varus than the uninjured leg (Table 2). There were definitive differences between the injured and uninjured legs from a coronal view of the single leg half squat (Fig. 3).

Comparing the gender differences in ACL-injured subjects, female subjects demonstrated significantly more external hip rotation and knee valgus in both the injured and uninjured legs than male subjects (Table 3). The characteristics of female ACL-injured subjects from the coronal view are shown in Fig. 4.

Discussion

The most important finding of the present study was the kinematic difference between the ACL-injured subjects and the healthy controls. The uninjured leg in female ACL-injured subjects exhibited less hip flexion and more external hip rotation and knee flexion compared with the dominant leg of the female controls. Less hip flexion in ACL-injured patients during single leg half squatting might suggest that they could perform more stable squatting by maintaining their hip in a more extended position.

Table 1 Comparison of mean joint angle at knee maximum flexion between control and uninjured knees of ACL-injured subjects during single leg squatting

Motion	Mean joint angle (°)					
	Male			Female		
	Control[a]	Subjects[b]	P values	Control[a]	Subjects[b]	P values
Knee						
Flexion	77.8 ± 11.7	74.3 ± 13.6	0.4783[n.s.]	66.2 ± 9.9	73.9 ± 13.3	0.0070[c]
Varus	16.9 ± 15.1	14.1 ± 11.5	0.6559[n.s.]	8.9 ± 8.2	3.7 ± 11.9	0.0814[n.s.]
External rotation	38.8 ± 12.6	18.9 ± 34.3	0.0090[c]	7.9 ± 49.3	14.1 ± 34.7	0.8147[n.s.]
Hip						
Flexion	35.7 ± 14.9	32.7 ± 17.3	0.3229[n.s.]	48.0 ± 11.3	29.9 ± 18.4	0.0000[c]
Varus	9.8 ± 12.0	12.0 ± 8.6	0.6166[n.s.]	8.3 ± 6.2	10.9 ± 8.7	0.1763[n.s.]
External rotation	5.5 ± 10.5	4.5 ± 8.6	0.3775[n.s.]	1.7 ± 6.1	9.1 ± 8.0	0.0000[c]

[a] Control group, dominant leg (14 males, 12 females)

[b] ACL deficient, uninjured leg (32 males, 31 females)

[c] Level of significance ($\alpha < 0.05$)
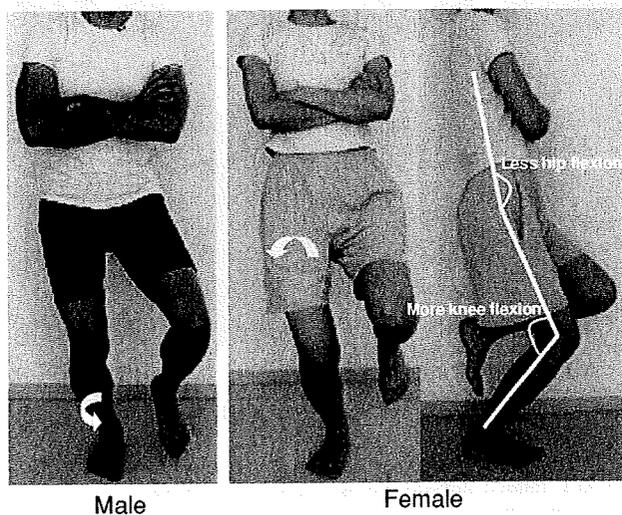

Male   Female

Fig. 2 Male ACL-injured subjects demonstrated less external knee rotation (arrow) than the control group. Female ACL-injured subjects demonstrated more external hip rotation (arrow), knee flexion, and less hip flexion than the control group

Several studies have examined the role of the hip joint as a potential risk factor in non-contact ACL injuries. Griffin et al. [8] reported the possibility of a decrease in hip-muscle activation influencing knee positioning. Zazulak et al. [24] showed that females exhibited a decrease in hip-muscle activity and an increase in rectus femoris muscle activity during landing maneuvers. According to Carcia et al. [25, 26], hip abductor fatigue altered frontal-plane lower extremity orientation; however, gluteus medius muscle activity was similar between genders during a drop jump. They also mentioned that gender differences in kinematics and electromyographic activity were present during the single leg squat for healthy

volunteers and concluded with an emphasis on the hip as a risk factor for ACL injury in female athletes [27].

In the current study, the hip joint of an ACL-injured female is possibly more unstable due to the dysfunction of hip muscles and/or structural disability of the hip joint, which is similar to that of acetabular dysplasia. It is difficult to maintain balance of the hip joint without dynamic stability during single leg squatting, because it has a multidirectional range of motion (flexion/extension, abduction/adduction, external rotation/internal rotation) due to its ball and socket structure. Assuming that the center of gravity of the body exists at the center of the pelvis and the center of the hip joint performing the single leg squat exists outside the center of gravity. Therefore, if dysfunction of the hip due to structural abnormality and/or muscle weakness occurs, it might be difficult for ACL-injured patients to maintain stability due to the characteristics of the multidirectional mobility of the hip joint. Previous research suggests that the gluteus medius plays an important role in controlling hip joint kinematics [7, 28]. However, gender differences in gluteus medius activity have not been well investigated, while rectus femoris activity was reportedly higher in females during a single leg squat [27]. Youdas et al. [29] reported that women are quadriceps dominant while performing a single leg squat. Anterior tibial translation is often caused by force applied by the quadriceps muscles [13, 30, 31]. For this reason, the ratio of the hamstring muscle strength to the quadriceps muscle strength is reportedly important as a risk factor of ACL injury.

There was an apparent difference of physical activity between female ACL-injured subjects and female controls. The significance of the difference should be considered. The results showed that female controls demonstrated more

Table 2 Differences of mean joint angle at knee maximum flexion between uninjured and injured knees in ACL-injured subjects during single leg squatting

Motion	Mean joint angle (°)					
	Male			Female		
	Uninjured	Injured	P values	Uninjured	Injured	P values
Knee						
Flexion	74.3 ± 13.6	64.7 ± 19.0	0.0004[a]	73.9 ± 13.3	68.8 ± 13.3	0.0645[n.s.]
Varus	14.1 ± 11.5	19.8 ± 11.3	0.0068[a]	3.7 ± 11.9	8.2 ± 12.5	0.0336[a]
External rotation	18.9 ± 34.3	1.6 ± 35.2	0.0079[a]	14.1 ± 34.7	6.8 ± 36.7	0.1948[n.s.]
Hip						
Flexion	32.7 ± 17.3	33.7 ± 17.7	0.7489[n.s.]	29.9 ± 18.4	33.6 ± 15.9	0.2428[n.s.]
Varus	12.0 ± 8.6	11.2 ± 8.5	0.5629[n.s.]	10.9 ± 8.7	13.6 ± 6.4	0.0760[n.s.]
External rotation	4.5 ± 8.6	1.4 ± 8.9	0.0369[a]	9.1 ± 8.0	7.7 ± 7.7	0.3442[n.s.]

[a] Level of significance ($\alpha < 0.05$)

Fig. 3 The injured leg of the male subjects demonstrated more internal and varus knee and more external hip rotation than the uninjured leg. The injured leg of the female subjects demonstrated more knee varus than the uninjured leg from the coronal view

Uninjured leg   Injured leg   Uninjured leg   Injured leg
Male   Female
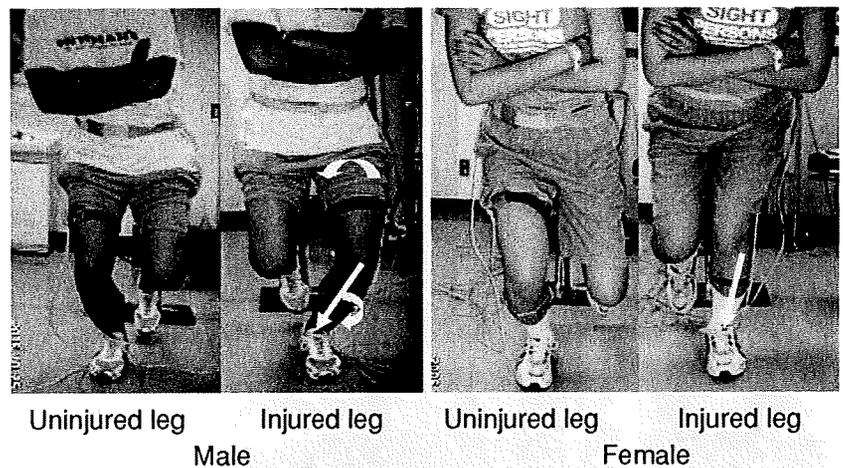
Table 3 Gender differences of mean joint angle at knee maximum flexion in ACL-injured subjects during single leg squatting

Motion	Mean joint angle (°)					
	Uninjured side			Injured side		
	Male	Female	P values	Male	Female	P values
Knee						
Flexion	74.3 ± 13.6	73.9 ± 13.3	0.8776[n.s.]	64.7 ± 19.0	68.8 ± 13.3	0.1265[n.s.]
Varus	14.1 ± 11.5	3.7 ± 11.9	0.0000[a]	19.8 ± 11.3	8.2 ± 12.5	0.0000[a]
External rotation	18.9 ± 34.3	14.1 ± 34.7	0.6674[n.s.]	1.6 ± 35.2	6.8 ± 36.7	0.4294[n.s.]
Hip						
Flexion	32.7 ± 17.3	29.9 ± 18.4	0.3744[n.s.]	33.7 ± 17.7	33.6 ± 15.9	0.9826[n.s.]
Varus	12.0 ± 8.6	10.9 ± 8.7	0.4381[n.s.]	11.2 ± 8.5	13.6 ± 6.4	0.1239[n.s.]
External rotation	4.5 ± 8.6	9.1 ± 8.0	0.0019[a]	1.4 ± 8.9	7.7 ± 7.7	0.0000[a]

[a] Level of significance ($\alpha < 0.05$)

hip flexion during the single leg squat, because they were well trained and might have participated in habitual squat exercise as physical training. The reason, the uninjured leg of female ACL-injured patients also demonstrated more hip extension compared with female controls, might have been due to the differences of the athletic level and kinds of

Fig. 4 Comparing gender differences from the coronal view, female ACL-injured subjects demonstrated significantly more external hip rotation and knee valgus in both the injured and uninjured legs than male subjects

Male   Female   Male   Female

Uninjured side   Injured side
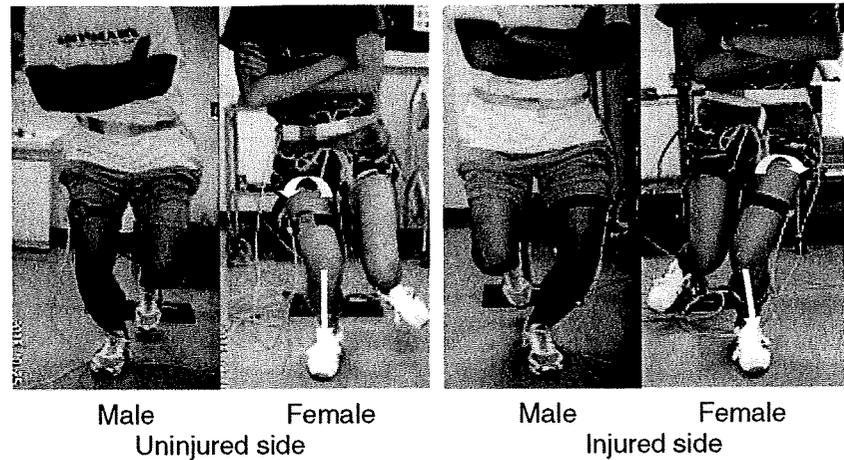
participating sports. However, the results were compatible with the study by Zeller et al. [27], which showed that female healthy subjects demonstrated more hip flexion than male healthy subjects despite greater rectus femoris muscle activation during single leg squatting. They discussed how quadriceps muscle could not be seen as the culprit in the lack of knee control and concluded that the activation patterns of the hip musculature were necessary. Thus, the effect of selection bias of the female controls was not clearly demonstrated in the current study.

The uninjured leg of male ACL-injured subjects did not exhibit any significant characteristics except for less external knee rotation compared to that of the male controls. The kinematic differences in the ACL-injured subjects shown in the study indicate the features of the ACL-injured leg.

In comparison with the ACL-injured and -uninjured side, the kinematics of the ACL-injured leg exhibited more knee varus than the uninjured leg in both male and female ACL-injured subjects. These results suggest that an ACL-injured knee could be maintained in balance more easily by maintaining a more varus position. The link between knee valgus loading and resultant increases in ACL strain has been demonstrated previously in vivo as well as in cadaveric studies [32, 33]. Therefore, for ACL-injured subjects, maintaining the knee position with more varus in ACL may have the potential for stabilizing an injured knee by reducing ACL dependency while maintaining the knee's position. This fact suggests that additional varus load stresses the ACL-injured knee.

In comparison with gender differences, the kinematics of female subjects exhibited more external hip rotation and knee valgus than male subjects in both the injured and uninjured legs. During landing, females often display valgus knee positioning. This position is defined as "dynamic valgus" and incorporates the movement of internal hip

rotation and adduction [14]. By studying the mechanism of non-contact ACL injuries, it was reported that there is a "position of no return", described as a loss of control at the hip and pelvis [7]. Hip and trunk-pelvis-hip control is assumed to be lost before ACL rupture. ACL rupture reportedly results from internal hip rotation and adduction, knee valgus, and external tibial rotation on a pronated, externally rotated foot. These gender differences in joint kinematics suggest that increased dynamic knee valgus contributes to ACL non-contact injury risk in women and that the hip plays an important role in controlling knee valgus.

With regard to hip adduction, no statistically significant difference was found between female and male subjects, even though females exhibited more knee valgus in comparison with males. This result raises the possibility that the female pelvis is inclined to the squatting leg side in order to adjust for the dysfunction of hip abductors. Therefore, the relative angle of hip adduction corresponding to the pelvis may have exhibited no statistical differences for male subjects.

Single leg squatting is considered to be a simple and safe clinical examination in comparison with single leg landing; therefore, it could be possible to perform it on many subjects at an out-patient clinic. In future studies, collecting kinematics data and comparing data of ACL-injured subjects from various kinds of sports and sports levels will be needed to establish the differences of knee kinematics during single leg half squatting and to develop an ACL-injury prevention method based on the results. The measurement setup can be also indicated to reveal the kinematic change in ACL-reconstructed knees. Such a study would reveal different kinematic characteristics of the ACL-reconstructed knees. Developing an adequate training regiment for each patient based on kinematics data will be important as rehabilitation immediately after ACL

Table 4 Comparison between uninjured and injured knees at angle of unified knee flexion in ACL-injured subjects during single leg squatting

Motion	Mean joint angle (°)					
	Male			Female		
	Uninjured	Injured	P values	Uninjured	Injured	P values
Knee						
Flexion[a]	63.9 ± 18.3	65.1 ± 17.9		66.5 ± 13.3	66.1 ± 12.2	
Varus	13.6 ± 10.5	20.7 ± 11.1	0.0052[b]	5.0 ± 12.2	7.2 ± 12.5	0.0486[b]
External rotation	12.1 ± 32.0	0.7 ± 36.8	0.0057[b]	21.4 ± 28.9	3.4 ± 34.8	$0.1142^{n.s.}$
Hip						
Flexion	29.9 ± 14.0	35.0 ± 17.1	$0.6185^{n.s.}$	28.1 ± 15.6	32.4 ± 16.8	$0.2973^{n.s.}$
Varus	9.7 ± 7.7	10.4 ± 8.6	$0.8681^{n.s.}$	10.3 ± 7.9	12.6 ± 7.5	$0.0984^{n.s.}$
External rotation	2.7 ± 8.4	1.0 ± 9.3	0.0419[b]	8.5 ± 7.6	7.5 ± 7.6	$0.4347^{n.s.}$

[a] An approximation of the ACL-injured leg's knee flexion angle was extracted from the data of the uninjured leg

[b] Level of significance ($\alpha < 0.05$)

reconstruction. Additional studies are needed to evaluate the relationship between hip morphology, general joint laxity, and other factors in female ACL-injured subjects.

Regarding study limitations, the first limitation of this study is that the knee flexion angle was not able to be controlled in either injured subjects or control subjects. An approximation of the injured leg's knee flexion angle was needed for extracting the knee flexion angle of the uninjured leg for unifying the knee flexion angle. We compared all the joint angles except the knee flexion angle in both the injured and uninjured legs. Comparing the injured and uninjured knees, the exact same results as shown in Table 4 were measured from the data at maximum knee flexion. Therefore, we considered the results to be valid for this study that evaluated hip and knee joint angles at maximum knee flexion while performing the single leg squat. The second limitation is that the physical activity of the female control group is higher than that of the male control group, and the sport item is very limited to the case of playing volleyball. This difference of physical activity and difference of sport item may have affected the results of kinematics of single leg squatting in the female control group.

Conclusion

Comparing the ACL-injured subjects with the healthy controls, female ACL-injured subjects demonstrated significantly more external hip rotation and knee flexion, and less hip flexion than the female controls during single leg half squatting, while male ACL-injured subjects did not exhibit any significant characteristics except for knee rotation.

The ACL-injured leg demonstrated more knee varus than the uninjured leg in both male and female ACL-injured

subjects. With regard to gender differences in ACL-injured subjects, female subjects demonstrated significantly more external hip rotation and knee valgus in both the injured and uninjured legs than male subjects.

The single leg half squat was a reproducible way to simplify the single leg landing position, and it was performed repeatedly and safely. Therefore, a large amount of data collection could be performed on many subjects.

References

1. Boden BP, Dean GS, Feagin JA, Garrett WE (2000) Mechanisms of anterior cruciate ligament injury. Orthopedics 23:573–578
2. McNair PJ, Marshall RN, Matheson JA (1990) Important features associated with acute anterior cruciate ligament injury. N Z Med J 103:537–539
3. Noyes FR, Mooar PA, Matthews DS, Butler DL (1983) The symptomatic anterior cruciate-deficient knee. 1. The long-term functional disability in athletically active individuals. J Bone Joint Surg Am 65:154–162
4. Arendt E, Dick R (1995) Knee injury patterns among men and women in collegiate basketball and soccer—NCAA data and review of literature. Am J Sports Med 23:694–701
5. Hewett TE, Myer GD, Ford KR (2006) Anterior cruciate ligament injuries in female athletes—part 1, mechanisms and risk factors. Am J Sports Med 34:299–311
6. Olsen OE, Myklebust G, Engebretsen L, Holme I, Bahr R (2003) Relationship between floor type and risk of ACL injury in team handball. Scand J Med Sci Sports 13:299–304
7. Ireland ML (1999) Anterior cruciate ligament injury in female athletes: epidemiology. J Athl Train 34:150–154
8. Griffin L, Agel J, Albohm M et al (2000) Non-contact anterior cruciate ligament injuries: risk factors and prevention strategies. J Am Acad Orthop Surg 8:141–150

9. Krosshaug T, Nakamae A, Boden BP (2007) Mechanisms of anterior cruciate ligament injury in basketball—video analysis of 39 cases. Am J Sports Med 35:359–367

10. Chappell JD, Limpisvasti O (2008) Effect of a neuromuscular training program on the kinetics and kinematics of jumping tasks. Am J Sports Med 36:1081–1086

11. Herman DC, Weinhold PS, Guskiewicz KM, Garrett WE, Yu B, Padua DA (2008) The effects of strength training on the lower extremity biomechanics of female recreational athletes during a stop-jump task. Am J Sports Med 36:733–740

12. Hewett TE, Ford KR, Myer GD (2006) Anterior cruciate ligament injuries in female athletes—part 2, a meta-analysis of neuromuscular interventions aimed at injury prevention. Am J Sports Med 34:490–498

13. Hewett TE, Lindenfeld TN, Riccobene JV, Noyes FR (1999) The effect of neuromuscular training on the incidence of knee injury in female athletes—a prospective study. Am J Sports Med 27:699–706

14. Hewett TE, Myer GD, Ford KR (2005) Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes. Am J Sports Med 33:492–501

15. Kernozek TW, Torry MR, Van Hoof H, Cowley H, Tanner S (2005) Gender differences in frontal and sagittal plane biomechanics during drop landings. Med Sci Sports Exerc 37:1003–1012

16. Lawrence RK, Kernozek TW, Miller EJ, Torry MR, Reuteman P (2008) Influences of hip external rotation strength on knee mechanics during single-leg drop landings in females. Clin Biomech 23:806–813

17. Lephart SM, Ferris CM, Riemann BL, Myers JB, Fu FH (2002) Gender differences in strength and lower extremity kinematics during landing. Clin Orthop Relat Res 401:162–169

18. Ortiz A, Olson S, Libby CL (2008) Landing mechanics between noninjured women and women with anterior cruciate ligament reconstruction during 2 jump tasks. Am J Sports Med 36:149–157

19. Pappas E, Hagins M, Sheikhzadeh A, Nordin M, Rose D (2007) Biomechanical differences between unilateral and bilateral landings from a jump: gender differences. Clin J Sport Med 17:263–268

20. Schmitz RJ, Kulas AS, Perrin DH, Riemann BL, Shultz SJ (2007) Sex differences in lower extremity biomechanics during single leg landings. Clin Biomech 22:681–688

21. Swartz EE, Decoster LC, Russell PJ, Croce RV (2005) Effects of developmental stage and sex on lower extremity kinematics and vertical ground reaction forces during landing. J Athl Train 40:9–14

22. Wilkerson GB, Colston MA, Shortt NI, Neal KL, Hoewischer PE, Pixley JJ (2004) Neuromuscular changes in female collegiate athletes resulting from a plyometric jump-training program. J Athl Train 39:17–23

23. Magit DP, McGarry M, Tibone JE, Lee TQ (2008) Comparison of cutaneous and transosseous electromagnetic position sensors in the assessment of tibial rotation in a cadaveric model. Am J Sports Med 36:971–977

24. Zazulak BT, Ponce PL, Straub SJ, Medvecky MJ, Avedisian L, Hewett TE (2005) Gender comparison of hip muscle activity during single-leg landing. J Orthop Sports Phys Ther 35(5):292–299

25. Carcia C, Eggen J, Shultz S (2005) Hip-abductor fatigue, frontal-plane landing angle, and excursion during a drop jump. J Sport Rehabil 14:321–331

26. Carcia CR, Martin RL (2007) The influence of gender on gluteus medius activity during a drop jump. Phys Ther in Sport 8:169–176

27. Zeller BL, McCrory JL, Ben Kibler W, Uhl TL (2003) Differences in kinematics and electromyographic activity between men and women during the single-legged squat. Am J Sports Med 31(3):449–456

28. Schmitz RJ, Riemann BL, Thompson T (2002) Gluteus medius activity during isometric closed-chain hip rotation. J Sport Rehabil 11:179–188

29. Youdas JW, Hollman JH, Hitchcock JR, Hoyme GJ, Johnsen JJ (2007) Comparison of hamstring and quadriceps femoris electromyographic activity between men and women during a single-limb squat on both a stable and labile surface. J Strength Cond Res 21:105–111

30. MacWilliams BA, Wilson DR, DesJardins JD, Romero J, Chao EYS (1999) Hamstrings cocontraction reduces internal rotation, anterior translation, and anterior cruciate ligament load in weight-bearing flexion. J Orthop Res 17:817–822

31. More RC, Karras BT, Neiman R, Fritschy D, Woo SLY, Daniel DM (1993) Hamstrings—an anterior cruciate ligament protagonist—an invitro study. Am J Sports Med 21:231–237

32. Lloyd DG, Buchanan TS (2001) Strategies of muscular support of varus and valgus isometric loads at the human knee. J Biomech 34:1257–1267

33. Markolf KL, Burchfield DI, Shapiro MM, Shepard ME, Finerman GAM, Slauterbeck JL (1995) Combined knee loading states that generate high anterior cruciate ligament forces. J Orthop Res 13:930–935

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Mesenchymal stem cell-based therapy for cartilage repair: a review

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Abstract Articular cartilage injury remains one of the major concerns in orthopaedic surgery. Mesenchymal stem cell (MSC) transplantation has been introduced to avoid some of the side effects and complications of current techniques. The purpose of this paper is to review the literature on MSC-based cell therapy for articular cartilage repair to determine if it can be an alternative treatment for cartilage injury. MSCs retain both high proliferative potential and multipotentiality, including chondrogenic differentiation potential, and a number of successful results in transplantation of MSCs into cartilage defects have been reported in animal studies. However, the use of MSCs for cartilage repair is still at the stage of preclinical and phase I studies, and no comparative clinical studies have been reported. Therefore, it is difficult to make conclusions in human studies. This requires randomized clinical trials to evaluate the effectiveness of MSC-based cell therapy for cartilage repair.

Keywords Mesenchymal stem cells · Cartilage repair ·
Cell transplantation · Chondrocytes

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Introduction

Cartilage defects have very limited intrinsic healing capacity. Partial thickness defects that do not penetrate the subchondral bone do not usually repair spontaneously [25], while repair of full thickness cartilage defects that penetrate the subchondral bone depends on the circumstances, such as age, defect size and location [17]. Small defects can repair spontaneously with production of hyaline cartilage, whereas larger defects will only repair with production of fibrous tissue or fibrocartilage which are biochemically and biomechanically different from normal hyaline cartilage. As a result, degeneration subsequently occurs which may progress to osteoarthritic change in some cases [91].

Various surgical methods have been proposed to regenerate articular cartilage. However, they all have inherent problems, leaving many patients with inadequately treated cartilage lesions. Recently, mesenchymal stem cells (MSCs) have been suggested as a source of cells for cell-based treatment of cartilage lesions. MSCs are known to play important roles in development, post-natal growth, repair and regeneration of mesenchymal tissues. They are easily isolated, and retain high expansion potential and multipotentiality that includes chondrogenic

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differentiation potential. Based on these properties, MSCs are potentially an attractive cell source for cartilage regenerative medicine. With regard to in vivo studies, transplantation of MSCs into full thickness articular cartilage defects has been attempted under various conditions. Although many successful results have been reported, a number of questions, such as from which tissue MSCs are suitable or what conditions are appropriate for cartilage repair, still exist, limiting clinical applications for cartilage injury. Currently, very few clinical studies of MSC transplantation for cartilage repair have been reported.

This paper briefly describes some of the problems associated with currently used methods for repair of cartilage lesions, followed by a review of the existing literature on MSC-based cell therapy for articular cartilage repair. We focus on three major parts in the process; transplanted cells, scaffolds and growth factors, and their differential performance in vitro and in MSC-based cell therapy in animal studies. Finally we review clinical studies on MSC therapy for cartilage repair, and discuss MSC therapy compared with autologous chondrocyte implantation (ACI), which is an established procedure worldwide [7, 10, 59, 62, 73]. The intention of this paper is to review whether MSC-based cell therapy can be an alternative for treatment of articular cartilage injury.

Current surgical treatment for articular cartilage injury

Bone marrow stimulation

Bone marrow stimulation is a technique in which subchondral bone is penetrated. One of the expectations for the penetration is to induce bone marrow-derived chondroprogenitors into the cartilage lesion. The procedure may also enhance the expression of cytokines to promote cartilage repair [57]. This technique includes drilling [75], abrasion [41] and microfracture [61, 93]. At present, these techniques are preferred by the majority of orthopaedic surgeons as they are easy to perform, need no special surgical instruments and are more cost effective than others [103]. However, cartilage defects only repair with fibrous tissue or fibrocartilage by these methods, probably because the number of chondroprogenitors induced from bone marrow is too small to promote repair with hyaline cartilage, and the results are often followed by degeneration in the repaired tissue [93].

Mosaicplasty

Mosaicplasty is a procedure whereby autologous osteochondral plugs are transplanted into the cartilage defect. By this method, the repaired tissue is predominantly composed of hyaline cartilage [33, 60]. However, donor site morbidity [78] and the limited availability of autologous osteochondral plugs limit the usefulness of this method, particularly for repair of large lesions.

Autologous chondrocyte implantation

The clinical use of ACI was first reported by Brittberg et al. in 1994 [10], following animal studies which had shown its effectiveness [29]. In this method, chondrocytes are obtained from a biopsy taken from a non-weight bearing part of the cartilage of the patients, and are expanded in vitro, followed by the injection of a suspension of expanded chondrocytes into cartilage defects, covered with autologous periosteal flap. Although clinical results of the original ACI were reported as promising [62, 73], this procedure has some potential disadvantages, such as leakage of transplanted cells, invasive surgical method, hypertrophy of periosteum [32, 50] and loss of chondrogenic phenotype of expanded chondrocytes in monolayer culture [8]. Second-generation ACI was introduced to improve these problems, and biomaterials such as collagen type I gel [69], hyaluronan-based scaffold [59] and collagen type I/III membrane [7] were applied to secure cells in the defect area, to restore chondrogenic phenotype by way of three-dimensional culture [28] and to replace the periosteum as defect coverage. At present, only two prospective studies comparing the original and second-generation ACI are available [7, 59] and both studies showed no significant differences in the short term clinical outcomes. As for the first generation ACI, the newly regenerated cartilage often consists of fibrous tissues [35, 96] possibly due to limited number of chondrocytes and their low proliferation potential. Bony overgrowth which causes thinning of the regenerated cartilage and violation of the tidemark is also of concern [1]. Moreover, this method still sacrifices healthy cartilage. These aspects limit ACI in the treatment of large defects and may increase the long-term risk of developing osteoarthritis.

Comparison of treatments currently in use for cartilage repair

Jakobsen et al. [40] evaluated quality and outcome of 69 clinical cartilage repair studies using microfracture, autologous osteochondral transplantation (mosaicplasty), autologous periosteal transplantation or ACI. Data from 3,987 surgical procedures in these studies were assessed. More than half of the studies were retrospective, and only four studies were prospective, randomized and controlled trials [35, 44]. No significant differences in outcome were found between the four techniques, and large variations in the reported outcomes were seen within each treatment

modality. The low-methodological quality found in these studies indicates that caution is required when interpreting results after surgical cartilage repair. It was concluded that firm recommendations on which procedure to choose cannot be provided at this time.

Mesenchymal stem cells

Friedenstein [23] first established the existence of MSCs and showed that bone marrow contains cells that can differentiate into bone and cartilage. His initial work has been extended by a number of investigators and it has been reported that MSCs isolated from bone marrow have self-renewal potential and multilineage differentiation potential including chondrogenesis [42, 74, 76, 81]. MSCs can be isolated from a variety of adult mesenchymal tissues, have extensive proliferation potential and are easily expanded without loss of their multilineage differentiation potential within several passages. Therefore, MSCs are perceived as an attractive cell source for regenerative medicine for cartilage injury.

Chondrogenesis of MSCs

Chondrogenesis of MSCs was first reported by Ashton et al. [5] and a defined medium for in vitro chondrogenesis of MSCs was first described by Johnstone et al. [42], who used micromass culture with transforming growth factor-beta (TGF-β) and dexamethasone. Sekiya et al. [85, 88] reported that addition of bone morphogenetic proteins (BMPs) enhanced chondrogenesis under the conditions employed by Johnstone et al. [42]. Currently, the micromass culture is widely used to evaluate chondrogenic potential of MSCs in vitro.

However, this in vitro chondrogenesis does not mimic cartilage formation during development. During micromass culture, MSCs increase expressions of both collagen type II (chondrocyte marker) and type X (hypertrophic chondrocyte marker) [6, 39]. Furthermore, MSCs continue to express collagen type I [94]. Other cytokines such as insulin-like growth factor (IGF) [71] and parathyroid hormone-related peptide (PTHrP) [43] have been tried for better differentiation cocktails, but it is still difficult to obtain in vitro MSC-based cartilage formation comparative with native cartilage tissue.

When bone marrow-derived mesenchymal cells were subcutaneously transplanted in special diffusion chambers, some cells differentiated into cartilage ectopically [5]. Also, MSCs implanted into osteochondral defects differentiated into chondrocytes [99]. On the other hand, after cartilage pellets differentiated from MSCs in vitro were

transplanted subcutaneously, these pellets disappeared [19] or calcified with vascular invasion [72]. This indicates the importance of signals from the microenvironment to induce cartilage formation of MSCs and to maintain its phenotypes [15, 47, 99].

MSCs from various mesenchymal tissues

Although bone marrow is considered to be a well-accepted source of MSCs, stem cells are present in a variety of mesenchymal tissues other than bone marrow and can be isolated from them, such as synovium [18], periosteum [24], skeletal muscle [11], adipose tissue [110], trabecular bone [82] and umbilical cord blood [55]. These MSCs are similar irrespective of their origin in that they have colony-forming ability and in vitro chondrogenic, osteogenic and adipogenic potentials [20, 30]. Recently, there are increasing number of studies describing the specific properties of MSCs, including chondrogenic potential, dependent on their origin [16, 37, 48, 56, 70, 77, 79, 83, 86, 105, 109].

Some comparative studies showed that MSCs from bone marrow had more in vitro chondrogenic potential than those from adipose tissue [16, 37, 56, 77, 79, 86, 105]. Sakaguchi et al. [83] harvested human bone marrow, synovium, periosteum, muscle and adipose tissue, and isolated and expanded MSCs in a similar condition. They demonstrated that MSCs derived from synovium had higher chondrogenic potential than those from other mesenchymal tissues. Yoshimura et al. [109] also demonstrated higher chondrogenic differentiation potential of MSCs from synovium in rats in a similar way.

One drawback of these studies is that the evaluation of in vitro chondrogenesis may not represent the chondrogenic potential of MSCs transplanted into cartilage defects. Park et al. [70] showed that MSCs from bone marrow and periosteum were superior to cells isolated from fat with respect to forming hyaline cartilaginous tissue when transplanted into cartilage defect in rats. Koga et al. [48] compared in vivo chondrogenic potential among various MSCs in rabbits and demonstrated that MSCs from synovium and bone marrow had a higher potential to repair cartilage defect than those from skeletal muscle and adipose tissue.

Which is a better MSC source for cartilage regeneration, bone marrow or synovium? Nimura et al. [67] reported that MSCs from synovium expanded much faster than those from bone marrow when cultured with autologous human serum. This is an advantage of MSCs from synovium. However, bone marrow is easier to harvest than synovium, which is a reason why bone marrow is more widely accepted as an MSC source.

Suitable conditions for cartilage repair with MSCs

Cell density

In ACI, chondrocyte density used for clinical treatment was 10^6 cells/ml or less when they chondrocytes embedded in a gel [7, 59, 69]. In MSC transplantation, higher density appears to be required. Koga et al. reported that 5×10^7 MSCs/ml embedded in collagen type I gel repaired cartilage defect successfully whereas 10^6 MSCs/ml resulted in failure in rabbit [48]. MSCs divided sparsely during in vitro [38, 87] and in vivo [47] chondrogenesis, whereas viable MSCs were decreased by apoptosis [38].

Growth and differentiation factors

Growth and differentiation factors including members of the TGF-β superfamily, IGF-1 or FGF have been shown to stimulate the chondrogenic differentiation of MSCs. Although some studies have reported that transplantation of undifferentiated MSCs into cartilage defects provided good results [1, 47, 99], these factors such as TGF-β [31, 54], BMPs [66], IGF-1[26] and, particularly, a combination of TGF-β and BMPs [88] have been shown to enhance cartilage repair in combination with MSCs in vitro and in animal studies. Such stimulations could be achieved by direct administration of recombinant growth and differentiation factors or by transfer of the respective genes, and each study showed that cartilage defects transplanted with enhanced MSCs led to better repair than those with untreated MSCs. These results suggest that the use of appropriate differentiation factors could improve cartilage repair by MSC transplantation. Presently, the differentiation factors cannot be ranked according to efficiency. There are some disadvantages with the use of growth factors. Direct administration into the injured site requires high dosages or repeated injections due to relatively short half-lives of these proteins in vivo. This technique is very expensive and may lead to unanticipated adverse effects [3, 98]. Gene therapy has been suggested as an alternative method by which these proteins can be provided for a prolonged period of time directly at the site of cell transplantation [27]. However, a large amount of work still has to be carried out to prove that gene therapy is sufficiently safe to allow clinical use [22]. Even if the growth factors are used only during in vitro differentiation, there are problems regarding availability of good manufacturing practice format, price and the fact that no combination of differentiation factors has been able to turn off collagen type I and collagen type X genes [6, 39, 94]. A better strategy might be to construct scaffolds which are able to deliver differentiation factors directly to the cells embedded in the scaffold. Currently some bioscaffolds exist that can elicit a controlled action and reaction to the surrounding tissue environment (bioactive), and others that exhibit a controlled chemical breakdown and resorption with ultimate replacement by regenerating tissue (resorbable). New generation biomaterials are also being designed to stimulate regeneration of living tissues using tissue engineering and in situ tissue regeneration methods. These materials will lead to limitless possibilities for cartilage regeneration [34, 108]. However, they require further investigations for clinical use.

Culture serum

MSCs have been expanded with foetal bovine serum (FBS) for research as well as for clinical use [36, 99]. However, supplementation with FBS has several risks, such as disease transmission and immune reaction [58, 89, 104]. Increasing the safety of MSC transplantation requires the use of safe and effective substitute for FBS. Autologous human serum has been investigated as a substitute for FBS. Several studies have compared the proliferative effects of autologous human serum and FBS on MSCs from bone marrow with variable results. Some reported that FBS was superior to human serum [52]. Others reported that their proliferative effects were similar [92, 106], and still others demonstrated that MSCs proliferated more in human serum than in FBS [45, 64, 90, 95]. The discrepancies among them may be from serum dose difference, from variations among donor sera, from differences in sample numbers and also from differences in harvested sites [2]. For other MSCs, Nimura et al. [67] reported that MSCs from synovium expanded more in human serum than in FBS through platelet-derived growth factor signalling, while opposite results were obtained with MSCs from bone marrow. As for chondrogenic potential, MSCs from bone marrow [90] and synovium [67] precultured in autologous human serum showed lower in vitro chondrogenic potential than in FBS, whereas in vivo chondrogenic potential of rabbit MSCs from synovium was similar [67].

Human platelet lysate (hPL) has also been reported as a substitute. Some reports showed that MSCs from bone marrow proliferated more in hPL than in FBS and retained their differentiation potentials including chondrogenesis, while there is a disadvantage that the amount of hPL obtained from a patient is less than half that of human serum [12, 13, 21, 53, 84]. Although there is no comparative study between human serum and hPL, they might be an effective and more beneficial substitute for FBS.

Clinical studies on MSC transplantation for cartilage repair

Presently only one prospective clinical study of MSC transplantation for cartilage repair has been published, in

Table 1 Clinical studies on MSC transplantation for cartilage repair

Authors	Year	No. of patients	Results and comments
Wakitani et al.	2002	24	Prospective, randomized study
			Bone marrow MSC versus cell-free scaffold
			No significance in clinical results
			Better arthroscopic and histological score in MSC group
Wakitani et al.	2004	2	Case reports transplanted in patellar defects
			Bone marrow MSC
			All are clinically improved
Wakitani et al.	2007	3	Three case reports involving nine defects in five knees
			Bone marrow MSC
			All are clinically improved
Kuroda et al.	2007	1	A case report
			Bone marrow MSC
			Clinically improved

which bone marrow-derived MSCs were resuspended in a collagen type I gel and transplanted with autologous periosteal flap [100]. In this study, patients with knee osteoarthritis who underwent a high tibial osteotomy were treated with a cell-containing scaffold with a periosteal flap transplanted into a cartilage defect in the medial femoral condyle and compared with patients who were transplanted with cell-free scaffold with a periosteal flap transplanted into a similar lesion. Although the cell-treated group showed no significant improvement clinically compared with the control group, the arthroscopic and histological score was better in MSC-transplanted group. There were also three case reports from the same group which reported that patients' clinical symptoms had improved [51, 101, 102] (Table 1). However, there is still no comparative clinical study with other surgical methods.

Comparison of chondrocyte and MSC transplantation for cartilage repair

As mentioned before, MSC transplantation has some advantages in cartilage repair over ACI. MSCs can be isolated from various tissues without harvesting healthy articular cartilage and are easily expanded without loss of their chondrogenic potential at early passages. Therefore, MSCs are an attractive alternative cell source not only for patients with focal cartilage lesion but also for those with osteoarthritis [65, 68]. However, there are also some disadvantages and risks to use MSCs for cartilage repair. Some reports showed that MSCs-derived chondrocytes expressed hypertrophy-related genes leading to cell death or calcification followed by vascularization when implanted subcutaneously or intramuscularly, whereas articular chondrocytes resisted calcification and vascular invasion [19]. When MSCs were transplanted into cartilage defects, while they could differentiate into chondrocytes according

to local microenvironment in articular joints, the thickness of the regenerated cartilage became thinner than the original thickness and the tidemark was violated [1, 47, 99]. Transformation of MSCs is also one of the concerns. It has been reported that MSCs can undergo spontaneous transformation after long-time culture [80, 97], although they can be managed safely during the standard ex vivo expansion period and such transformation is considered to be very rare [9]. Further investigation to solve these problems is required.

A few animal studies have been published comparing ACI and MSC transplantation for cartilage repair. In these studies, no significant differences were observed in histological score between the group transplanted with chondrocytes and with MSCs [1, 107], although repaired tissue with MSCs appeared better in cell arrangement, subchondral bone remodelling, and integration with surrounding cartilage [107].

Less invasive technique for MSC transplantation

Treatment with MSCs (and chondrocytes) requires the transplantation of a cell and scaffold composite. If periosteal coverage is needed, the method is quite invasive as it requires harvesting the periosteum and fixation with suturing to the neighbouring cartilage. Moreover, hypertrophy and ossification are of concern [32, 50, 69]. Without periosteum, a scaffold is needed to keep the cells at the injured site; the scaffolds are derived from animals, thereby increasing the risk of disease transmission and immune reaction [14]. The easiest and the least invasive method might be intra-articular injection; however, with this technique, most of the injected cells adhered to synovial tissues [63], which might increase the risk of adverse effects such as synovial proliferation, and only a small portion of the cells adhered to the cartilage defects [49]. Recently, some

papers reported less invasive methods without scaffolds to adhere transplanted cells effectively [4, 46, 49]. If clinically successful, such methods may extend the indications for MSC-based cell therapy for cartilage repair.

Conclusions

As articular cartilage defects have very limited intrinsic healing capacity, development of new methods for treatment for cartilage defects is of major importance for orthopaedic surgeons. Although various surgical methods have been attempted, including bone marrow stimulation technique, mosaicplasty and ACI, each of them has some disadvantages. MSC-based cell therapy is expected to be an alternative for cartilage repair because MSCs are easily isolated from a variety of mesenchymal tissues, have high proliferative potential and have chondrogenic potential. In animal studies, transplantation of MSCs into cartilage defects has been attempted under different circumstances, and a number of publications exist on cell source, cell density, growth and differentiation factors, culture serum and scaffold. Recently, less invasive techniques for MSC transplantation into cartilage defects have also been developed, and the clinical use of MSCs may well have a bright future. However, the technique is still in the state of preclinical and phase I studies, and there is no comparative clinical study with other surgical methods. Moreover, some concerns still exist about the chondrogenesis and genetic stability of MSCs. Randomized clinical trials are needed to evaluate the effectiveness of MSC-based cell therapy for cartilage repair.

References

1. Adachi N, Sato K, Usas A, Fu FH, Ochi M, Han CW, Niyibizi C, Huard J (2002) Muscle derived, cell based ex vivo gene therapy for treatment of full thickness articular cartilage defects. J Rheumatol 29:1920–1930
2. Akintoye SO, Lam T, Shi S, Brahim J, Collins MT, Robey PG (2006) Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. Bone 38:758–768. doi:10.1016/j.bone.2005.10.027
3. Allen JB, Manthey CL, Hand AR, Ohura K, Ellingsworth L, Wahl SM (1990) Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta. J Exp Med 171:231–247. doi:10.1084/jem.171.1.231
4. Ando W, Tateishi K, Hart DA, Katakai D, Tanaka Y, Nakata K, Hashimoto J, Fujie H, Shino K, Yoshikawa H, Nakamura N (2007) Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. Biomaterials 28:5462–5470. doi:10.1016/j.biomaterials.2007.08.030
5. Ashton BA, Allen TD, Howlett CR, Eaglesom CC, Hattori A, Owen M (1980) Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. Clin Orthop Relat Res 313:294–307
6. Barry F, Boynton RE, Liu B, Murphy JM (2001) Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. Exp Cell Res 268:189–200. doi:10.1006/excr.2001.5278
7. Bartlett W, Skinner JA, Gooding CR, Carrington RW, Flanagan AM, Briggs TW, Bentley G (2005) Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg Br 87:640–645. doi:10.1302/0301-620X.87B5.15905
8. Benya PD, Shaffer JD (1982) Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell 30:215–224. doi:10.1016/0092-8674(82)90027-7
9. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F (2007) Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res 67:9142–9149. doi:10.1158/0008-5472.CAN-06-4690
10. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 331:889–895. doi:10.1056/NEJM199410063311401
11. Cao B, Zheng B, Jankowski RJ, Kimura S, Ikezawa M, Deasy B, Cummins J, Epperly M, Qu-Petersen Z, Huard J (2003) Muscle stem cells differentiate into haematopoietic lineages but retain myogenic potential. Nat Cell Biol 5:640–646. doi:10.1038/ncb1008
12. Capelli C, Domenghini M, Borleri G, Bellavita P, Poma R, Carobbio A, Mico C, Rambaldi A, Golay J, Introna M (2007) Human platelet lysate allows expansion and clinical grade production of mesenchymal stromal cells from small samples of bone marrow aspirates or marrow filter washouts. Bone Marrow Transplant 40:785–791. doi:10.1038/sj.bmt.1705798
13. Carrancio S, Lopez-Holgado N, Sanchez-Guijo FM, Villaron E, Barbado V, Tabera S, Diez-Campelo M, Blanco J, San Miguel JF, Del Canizo MC (2008) Optimization of mesenchymal stem cell expansion procedures by cell separation and culture conditions modification. Exp Hematol 36:1014–1021. doi:10.1016/j.exphem.2008.03.012
14. Charriere G, Bejot M, Schnitzler L, Ville G, Hartmann DJ (1989) Reactions to a bovine collagen implant. Clinical and immunologic study in 705 patients. J Am Acad Dermatol 21:1203–1208. doi:10.1016/S0190-9622(89)70330-3
15. Chen J, Wang C, Lu S, Wu J, Guo X, Duan C, Dong L, Song Y, Zhang J, Jing D, Wu L, Ding J, Li D (2005) In vivo chondrogenesis of adult bone-marrow-derived autologous mesenchymal stem cells. Cell Tissue Res 319:429–438. doi:10.1007/s00441-004-1025-0
16. Colter DC, Sekiya I, Prockop DJ (2001) Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc Natl Acad Sci USA 98:7841–7845. doi:10.1073/pnas.141221698
17. Convery FR, Akeson WH, Keown GH (1972) The repair of large osteochondral defects. An experimental study in horses. Clin Orthop Relat Res 82:253–262. doi:10.1097/00003086-197201000-00033
18. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP (2001) Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 44:1928–1942. doi:10.1002/1529-0131(200108)44:8<1928::AID-ART331>3.0.CO;2-P
19. De Bari C, Dell'Accio F, Luyten FP (2004) Failure of in vitro-differentiated mesenchymal stem cells from the synovial

membrane to form ectopic stable cartilage in vivo. Arthritis Rheum 50:142–150. doi:10.1002/art.11450

20. De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser J, Hedrick MH (2003) Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs 174:101–109. doi:10.1159/000071150

21. Doucet C, Ernou I, Zhang Y, Llense JR, Begot L, Holy X, Lataillade JJ (2005) Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications. J Cell Physiol 205:228–236. doi:10.1002/jcp.20391

22. Evans CH, Ghivizzani SC, Robbins PD (2006) Gene therapy for arthritis: what next? Arthritis Rheum 54:1714–1729. doi:10.1002/art.21886

23. Friedenstein AJ, Gorskaja JF, Kulagina NN (1976) Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4:267–274

24. Fukumoto T, Sperling JW, Sanyal A, Fitzsimmons JS, Reinholz GG, Conover CA, O'Driscoll SW (2003) Combined effects of insulin-like growth factor-1 and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis in vitro. Osteoarthritis Cartilage 11:55–64. doi:10.1053/joca.2002.0869

25. Fuller JA, Ghadially FN (1972) Ultrastructural observations on surgically produced partial-thickness defects in articular cartilage. Clin Orthop Relat Res 86:193–205. doi:10.1097/00003086-197207000-00031

26. Gelse K, von der Mark K, Aigner T, Park J, Schneider H (2003) Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. Arthritis Rheum 48:430–441. doi:10.1002/art.10759

27. Gelse K, Schneider H (2006) Ex vivo gene therapy approaches to cartilage repair. Adv Drug Deliv Rev 58:259–284. doi:10.1016/j.addr.2006.01.019

28. Gigante A, Bevilacqua C, Ricevuto A, Mattioli-Belmonte M, Greco F (2007) Membrane-seeded autologous chondrocytes: cell viability and characterization at surgery. Knee Surg Sports Traumatol Arthrosc 15:88–92. doi:10.1007/s00167-006-0115-9

29. Grande DA, Pitman MI, Peterson L, Menche D, Klein M (1989) The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res 7:208–218. doi:10.1002/jor.1100070208

30. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM (2001) Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 189:54–63. doi:10.1002/jcp.1138

31. Guo X, Zheng Q, Yang S, Shao Z, Yuan Q, Pan Z, Tang S, Liu K, Quan D (2006) Repair of full-thickness articular cartilage defects by cultured mesenchymal stem cells transfected with the transforming growth factor beta1 gene. Biomed Mater 1:206–215. doi:10.1088/1748-6041/1/4/006

32. Haddo O, Mahroof S, Higgs D, David L, Pringle J, Bayliss M, Cannon SR, Briggs TW (2004) The use of chondrogide membrane in autologous chondrocyte implantation. Knee 11:51–55. doi:10.1016/S0968-0160(03)00041-3

33. Hangody L, Feczko P, Bartha L, Bodo G, Kish G (2001) Mosaicplasty for the treatment of articular defects of the knee and ankle. Clin Orthop Relat Res S328–S336. doi:10.1097/00003086-200110001-00030

34. Hench LL, Polak JM (2002) Third-generation biomedical materials. Science 295:1014–1017. doi:10.1126/science.1067404

35. Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R (2003) Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. J Bone Joint Surg Am 85-A:185–192

36. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T (2002) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. Proc Natl Acad Sci USA 99:8932–8937. doi:10.1073/pnas.132252399

37. Huang JI, Kazmi N, Durbhakula MM, Hering TM, Yoo JU, Johnstone B (2005) Chondrogenic potential of progenitor cells derived from human bone marrow and adipose tissue: a patient-matched comparison. J Orthop Res 23:1383–1389

38. Ichinose S, Tagami M, Muneta T, Sekiya I (2005) Morphological examination during in vitro cartilage formation by human mesenchymal stem cells. Cell Tissue Res 322:217–226. doi:10.1007/s00441-005-1140-6

39. Ichinose S, Yamagata K, Sekiya I, Muneta T, Tagami M (2005) Detailed examination of cartilage formation and endochondral ossification using human mesenchymal stem cells. Clin Exp Pharmacol Physiol 32:561–570

40. Jakobsen RB, Engebretsen L, Slauterbeck JR (2005) An analysis of the quality of cartilage repair studies. J Bone Joint Surg Am 87:2232–2239. doi:10.2106/JBJS.D.02904

41. Johnson LL (1986) Arthroscopic abrasion arthroplasty historical and pathologic perspective: present status. Arthroscopy 2:54–69. doi:10.1016/S0749-8063(86)80012-3

42. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU (1998) In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 238:265–272. doi:10.1006/excr.1997.3858

43. Kim YJ, Kim HJ, Im GI (2008) PTHrP promotes chondrogenesis and suppresses hypertrophy from both bone marrow-derived and adipose tissue-derived MSCs. Biochem Biophys Res Commun 373:104–108. doi:10.1016/j.bbrc.2008.05.183

44. Knutsen G, Engebretsen L, Ludvigsen TC, Drogset JO, Grontvedt T, Solheim E, Strand T, Roberts S, Isaksen V, Johansen O (2004) Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J Bone Joint Surg Am 86-A:455–464

45. Kobayashi T, Watanabe H, Yanagawa T, Tsutsumi S, Kayakabe M, Shinozaki T, Higuchi H, Takagishi K (2005) Motility and growth of human bone-marrow mesenchymal stem cells during ex vivo expansion in autologous serum. J Bone Joint Surg Br 87:1426–1433. doi:10.1302/0301-620X.87B10.16160

46. Kobayashi T, Ochi M, Yanada S, Ishikawa M, Adachi N, Deie M, Arihiro K (2008) A novel cell delivery system using magnetically labeled mesenchymal stem cells and an external magnetic device for clinical cartilage repair. Arthroscopy 24:69–76. doi:10.1016/j.arthro.2007.08.017

47. Koga H, Muneta T, Ju YJ, Nagase T, Nimura A, Mochizuki T, Ichinose S, von der Mark K, Sekiya I (2007) Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. Stem Cells 25:689–696. doi:10.1634/stemcells.2006-0281

48. Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I (2008) Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res 333:207–215. doi:10.1007/s00441-008-0633-5

49. Koga H, Shimaya M, Muneta T, Nimura A, Morito T, Hayashi M, Suzuki S, Ju YJ, Mochizuki T, Sekiya I (2008) Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. Arthritis Res Ther 10:R84. doi:10.1186/ar2460

50. Kreuz PC, Steinwachs M, Erggelet C, Krause SJ, Ossendorf C, Maier D, Ghanem N, Uhl M, Haag M (2007) Classification of graft hypertrophy after autologous chondrocyte implantation

of full-thickness chondral defects in the knee. Osteoarthritis Cartilage 15:1339–1347. doi:10.1016/j.joca.2007.04.020

51. Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, Ohgushi H, Wakitani S, Kurosaka M (2007) Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. Osteoarthritis Cartilage 15:226–231. doi:10.1016/j.joca.2006.08.008

52. Kuznetsov SA, Mankani MH, Robey PG (2000) Effect of serum on human bone marrow stromal cells: ex vivo expansion and in vivo bone formation. Transplantation 70:1780–1787. doi:10.1097/00007890-200012270-00018

53. Lange C, Cakiroglu F, Spiess AN, Cappallo-Obermann H, Dierlamm J, Zander AR (2007) Accelerated and safe expansion of human mesenchymal stromal cells in animal serum-free medium for transplantation and regenerative medicine. J Cell Physiol 213:18–26. doi:10.1002/jcp.21081

54. Lee KH, Song SU, Hwang TS, Yi Y, Oh IS, Lee JY, Choi KB, Choi MS, Kim SJ (2001) Regeneration of hyaline cartilage by cell-mediated gene therapy using transforming growth factor beta 1-producing fibroblasts. Hum Gene Ther 12:1805–1813. doi:10.1089/104303401750476294

55. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH (2004) Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 103:1669–1675. doi:10.1182/blood-2003-05-1670

56. Liu TM, Martina M, Hutmacher DW, Hui JH, Lee EH, Lim B (2007) Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. Stem Cells 25:750–760. doi:10.1634/stemcells.2006-0394

57. Lotz M (2001) Cytokines in cartilage injury and repair. Clin Orthop Relat Res S108–S115. doi:10.1097/00003086-20011 0001-00011

58. Mackensen A, Drager R, Schlesier M, Mertelsmann R, Lindemann A (2000) Presence of IgE antibodies to bovine serum albumin in a patient developing anaphylaxis after vaccination with human peptide-pulsed dendritic cells. Cancer Immunol Immunother 49:152–156. doi:10.1007/s002620050614

59. Manfredini M, Zerbinati F, Gildone A, Faccini R (2007) Autologous chondrocyte implantation: a comparison between an open periosteal-covered and an arthroscopic matrix-guided technique. Acta Orthop Belg 73:207–218

60. Matsusue Y, Yamamuro T, Hama H (1993) Arthroscopic multiple osteochondral transplantation to the chondral defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy 9:318–321. doi:10.1016/S0749-8063(05)80428-1

61. Minas T, Nehrer S (1997) Current concepts in the treatment of articular cartilage defects. Orthopedics 20:525–538

62. Minas T (2001) Autologous chondrocyte implantation for focal chondral defects of the knee. Clin Orthop Relat Res S349–S361. doi:10.1097/00003086-200110001-00032

63. Mizuno K, Muneta T, Morito T, Ichinose S, Koga H, Nimura A, Mochizuki T, Sekiya I (2008) Exogenous synovial stem cells adhere to defect of meniscus and differentiate into cartilage cells. J Med Dent Sci 55:101–111

64. Mizuno N, Shiba H, Ozeki Y, Mouri Y, Niitani M, Inui T, Hayashi H, Suzuki K, Tanaka S, Kawaguchi H, Kurihara H (2006) Human autologous serum obtained using a completely closed bag system as a substitute for foetal calf serum in human mesenchymal stem cell cultures. Cell Biol Int 30:521–524. doi:10.1016/j.cellbi.2006.01.010

65. Murphy JM, Fink DJ, Hunziker EB, Barry FP (2003) Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 48:3464–3474. doi:10.1002/art.11365

66. Nawata M, Wakitani S, Nakaya H, Tanigami A, Seki T, Nakamura Y, Saito N, Sano K, Hidaka E, Takaoka K (2005) Use

of bone morphogenetic protein 2 and diffusion chambers to engineer cartilage tissue for the repair of defects in articular cartilage. Arthritis Rheum 52:155–163. doi:10.1002/art.20713

67. Nimura A, Muneta T, Koga H, Mochizuki T, Suzuki K, Makino H, Umezawa A, Sekiya I (2008) Increased proliferation of human synovial mesenchymal stem cells with autologous human serum: comparisons with bone marrow mesenchymal stem cells and with fetal bovine serum. Arthritis Rheum 58:501–510. doi:10.1002/art.23219

68. Noth U, Steinert AF, Tuan RS (2008) Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. Nat Clin Pract Rheumatol 4:371–380

69. Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J (2002) Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. J Bone Joint Surg Br 84:571–578. doi:10.1302/0301-620X.84B4.11947

70. Park J, Gelse K, Frank S, von der Mark K, Aigner T, Schneider H (2006) Transgene-activated mesenchymal cells for articular cartilage repair: a comparison of primary bone marrow-, perichondrium/periosteum- and fat-derived cells. J Gene Med 8:112–125. doi:10.1002/jgm.826

71. Pei M, He F, Vunjak-Novakovic G (2008) Synovium-derived stem cell-based chondrogenesis. Differentiation

72. Pelttari K, Winter A, Steck E, Goetzke K, Hennig T, Ochs BG, Aigner T, Richter W (2006) Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. Arthritis Rheum 54:3254–3266. doi:10.1002/art.22136

73. Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A (2000) Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res 212–234. doi:10.1097/00003086-200005000-00020

74. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147. doi:10.1126/science.284.5411.143

75. Pridie KH (1959) A method of resurfacing osteoarthritic knee joints. J Bone Joint Surg Br 41:618–619

76. Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276:71–74. doi:10.1126/science.276.5309.71

77. Prockop DJ, Gregory CA, Spees JL (2003) One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci USA 100(Suppl 1):11917–11923. doi:10.1073/pnas.1834138100

78. Reddy S, Pedowitz DI, Parekh SG, Sennett BJ, Okereke E (2007) The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the talus. Am J Sports Med 35:80–85. doi:10.1177/0363546506290986

79. Rider DA, Dombrowski C, Sawyer AA, Ng GH, Leong D, Hutmacher DW, Nurcombe V, Cool SM (2008) Autocrine fibroblast growth factor 2 increases the multipotentiality of human adipose-derived mesenchymal stem cells. Stem Cells 26:1598–1608. doi:10.1634/stemcells.2007-0480

80. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A (2005) Spontaneous human adult stem cell transformation. Cancer Res 65:3035–3039

81. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131:324–336. doi:10.1016/j.cell.2007.08.025

82. Sakaguchi Y, Sekiya I, Yagishita K, Ichinose S, Shinomiya K, Muneta T (2004) Suspended cells from trabecular bone by

collagenase digestion become virtually identical to mesenchymal stem cells obtained from marrow aspirates. Blood 104:2728–2735. doi:10.1182/blood-2003-12-4452

83. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T (2005) Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 52:2521–2529. doi:10.1002/art.21212

84. Schallmoser K, Bartmann C, Rohde E, Reinisch A, Kashofer K, Stadelmeyer E, Drexler C, Lanzer G, Linkesch W, Strunk D (2007) Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells. Transfusion 47:1436–1446. doi:10.1111/j.1537-2995.2007.01220.x

85. Sekiya I, Colter DC, Prockop DJ (2001) BMP-6 enhances chondrogenesis in a subpopulation of human marrow stromal cells. Biochem Biophys Res Commun 284:411–418. doi:10.1006/bbrc.2001.4898

86. Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ (2002) Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells 20:530–541. doi:10.1634/stemcells.20-6-530

87. Sekiya I, Vuoristo JT, Larson BL, Prockop DJ (2002) In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. Proc Natl Acad Sci USA 99:4397–4402. doi:10.1073/pnas.052716199

88. Sekiya I, Larson BL, Vuoristo JT, Reger RL, Prockop DJ (2005) Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. Cell Tissue Res 320:269–276. doi:10.1007/s00441-004-1075-3

89. Selvaggi TA, Walker RE, Fleisher TA (1997) Development of antibodies to fetal calf serum with arthus-like reactions in human immunodeficiency virus-infected patients given syngeneic lymphocyte infusions. Blood 89:776–779

90. Shahdadfar A, Fronsdal K, Haug T, Reinholt FP, Brinchmann JE (2005) In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cells 23:1357–1366. doi:10.1634/stemcells.2005-0094

91. Shelbourne KD, Jari S, Gray T (2003) Outcome of untreated traumatic articular cartilage defects of the knee: a natural history study. J Bone Joint Surg Am 85:8–16

92. Spees JL, Gregory CA, Singh H, Tucker HA, Peister A, Lynch PJ, Hsu SC, Smith J, Prockop DJ (2004) Internalized antigens must be removed to prepare hypoimmunogenic mesenchymal stem cells for cell and gene therapy. Mol Ther 9:747–756. doi:10.1016/j.ymthe.2004.02.012

93. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG (2003) Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy 19:477–484. doi:10.1053/jars.2003.50112

94. Steck E, Bertram H, Abel R, Chen B, Winter A, Richter W (2005) Induction of intervertebral disc-like cells from adult mesenchymal stem cells. Stem Cells 23:403–411. doi:10.1634/stemcells.2004-0107

95. Stute N, Holtz K, Bubenheim M, Lange C, Blake F, Zander AR (2004) Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. Exp Hematol 32:1212–1225. doi:10.1016/j.exphem.2004.09.003

96. Tins BJ, McCall IW, Takahashi T, Cassar-Pullicino V, Roberts S, Ashton B, Richardson J (2005) Autologous chondrocyte implantation in knee joint: MR imaging and histologic features at 1-year follow-up. Radiology 234:501–508. doi:10.1148/radiol.2342031970

97. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, Bell S, Xia L, Zhou N, Riddle M, Schroeder TM, Westendorf JJ, McIvor RS, Hogendoorn PC, Szuhai K, Oseth L, Hirsch B, Yant SR, Kay MA, Peister A, Prockop DJ, Fibbe WE, Blazar BR (2007) Sarcoma derived from cultured mesenchymal stem cells. Stem Cells 25:371–379. doi:10.1634/stemcells.2005-0620

98. van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB (1998) Differential effects of local application of BMP-2 or TGF-beta 1 on both articular cartilage composition and osteophyte formation. Osteoarthritis Cartilage 6:306–317. doi:10.1053/joca.1998.0129

99. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, Goldberg VM (1994) Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 76:579–592

100. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M (2002) Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 10:199–206. doi:10.1053/joca.2001.0504

101. Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S (2004) Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. Cell Transplant 13:595–600. doi:10.3727/000000004783983747

102. Wakitani S, Nawata M, Tensho K, Okabe T, Machida H, Ohgushi H (2007) Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. J Tissue Eng Regen Med 1:74–79. doi:10.1002/term.8

103. Wakitani S, Kawaguchi A, Tokuhara Y, Takaoka K (2008) Present status of and future direction for articular cartilage repair. J Bone Miner Metab 26:115–122. doi:10.1007/s00774-007-0802-8

104. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG (1996) A new variant of Creutzfeldt–Jakob disease in the UK. Lancet 347:921–925. doi:10.1016/S0140-6736(96)91412-9

105. Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W (2003) Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum 48:418–429. doi:10.1002/art.10767

106. Yamamoto N, Isobe M, Negishi A, Yoshimasu H, Shimokawa H, Ohya K, Amagasa T, Kasugai S (2003) Effects of autologous serum on osteoblastic differentiation in human bone marrow cells. J Med Dent Sci 50:63–69

107. Yan H, Yu C (2007) Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. Arthroscopy 23:178–187. doi:10.1016/j.arthro.2006.09.005

108. Yang W, Gomes RR, Brown AJ, Burdett AR, Alicknavitch M, Farach-Carson MC, Carson DD (2006) Chondrogenic differentiation on perlecan domain I, collagen II, and bone morphogenetic protein-2-based matrices. Tissue Eng 12:2009–2024. doi:10.1089/ten.2006.12.2009

109. Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I (2007) Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell Tissue Res 327:449–462. doi:10.1007/s00441-006-0308-z

110. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295. doi:10.1091/mbc.E02-02-0105

In Vivo Pharmacokinetics of Ketoprofen after Patch Application in the Mexican Hairless Pig

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ABSTRACT: To evaluate the pharmacokinetics of topical drugs, *in vitro* permeation studies are performed using sacrificed pig skin or human tissues resected at surgery; however, these methods have their limitations in *in vivo* pharmacokinetics. This study examined the usefulness of Mexican hairless pigs for *in vivo* pharmacokinetic study, especially the drug concentration in the tissues. A ketoprofen patch was applied on the back of Mexican hairless pigs for 24 h, followed by sequential collection of blood specimens from 0 to 36 h ($n = 3$). Also, the skin, subcutaneous fat, fascia and muscle from the center of the site of application were excised at 12 h after the application ($n = 4$). Ketoprofen was first detected in the plasma at 8 h, the concentration increasing up to 24 h; the plasma concentration began to decrease after the removal of the ketoprofen patch. Ketoprofen concentrations in the tissues decreased with increasing depth of the tissues, but the values in the deep muscles, being the lowest among the tissues examined, were still higher than those in the plasma. While the data of drug concentration in human tissue are difficult to test, the Mexican hairless pig model appears to be attractive for *in vivo* pharmacokinetic studies of topically applied ketoprofen. Copyright © 2009 John Wiley & Sons, Ltd.

Key words: ketoprofen; Mexican hairless pig; patch; pharmacokinetics; topical application

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used widely for pain relief in musculoskeletal disorders [1]. Although oral formulations of NSAIDs are currently popular, they are associated with a high incidence of adverse effects, including stomach irritation, hepatotoxicity and

kidney failure [2,3]. In order to minimize the incidence of systemic events related to oral formulations of NSAIDs, topical forms of the drugs have been developed and the ketoprofen patch is one such product [4].

To evaluate the pharmacokinetics of topically applied drugs, *in vitro* permeation studies [5,6] and *in vivo* pharmacokinetic studies have been performed using small animals [7]; however, the results obtained from such studies may not be directly applicable to humans. There have been several reports describing the NSAID concentrations in human tissues after transdermal

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application [8–11], but the information from these studies remains limited. Larger animals will be preferable for *in vivo* pharmacokinetic studies for obtaining results applicable to humans.

Miniature pigs are used widely for medical research because of their easy handling and raising [12]. Among miniature pigs, it was considered that hairless pigs might be highly suitable for the evaluation of topical drugs, because the structure of the hairless pig skin is similar to that of human [13]. This study evaluated the *in vivo* pharmacokinetics of ketoprofen applied topically on the back of Mexican hairless pigs. The results demonstrated the usefulness of the Mexican hairless pig for the study of NSAID patches.

Materials and Methods

Animals

To collect sequential blood specimens, three Mexican hairless pigs [14] (National Livestock Breeding Center, Ibaraki, Japan) aged 12 months old and weighing 23.1–38.0 kg were used. To obtain en bloc tissue specimens from the back, four Mexican hairless pigs aged 5–22 months old and weighing 9.8–30.8 kg were used. All Mexican hairless pigs used were bred under specific pathogen-free (SPF) conditions. All the pigs had free access during the study period to food and water in a postoperative care cage which was 40 cm in width, 121 cm in depth, and 109 cm in height. The schemes of the animal experiments had been investigated and permitted by The Judging Committee of Experimental Animal Ethics of Jichi Medical University.

Topical application of ketoprofen

The sparse hairs on the back of the animals were shaved with an electrical clipper and the application area was swept with dry cotton. The ketoprofen patch (10 cm × 7 cm; Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan) containing 20 mg of ketoprofen was applied on the back of the Mexican hairless pigs. The medial margin of the patch was located at 3 cm to the left of the spinous processes of the thoracic vertebrae.

Plasma preparation

One day before the experiment, a central venous catheter was placed in the right medial cervical vein in all the animals under general anesthesia for blood sampling. Five milliliter blood samples were collected in heparinized syringes at 0, 1, 2, 4, 6, 8, 12 and 24 h, after which the ketoprofen patch was removed; thereafter, blood samples were collected again at 28, 32 and 36 h. The collected blood samples were immediately centrifuged at 3000 rpm for 15 min. Plasma was separated and the plasma samples were cryopreserved at −20°C until analysis.

Tissue sampling

The ketoprofen patches were applied on the backs of the animals for 12 h. Immediately after removal of the patches and wiping off of the drug remaining on the skin surface with wet cotton, the skin, subcutaneous fat, fascia and muscle at the center of the patch application site were excised en bloc (2 cm × 2 cm × 3 cm) under anesthesia induced by intramuscular injection of 10 mg/kg of ketamine, 2 mg/kg of xylazine and 0.02 mg/kg of atropine. The specimens were then divided into five sections; skin, subcutaneous fat, fascia, superficial muscle up to 5 mm thickness, and the remaining deeper muscle. All specimens were cryopreserved at −20°C until the analysis.

Ketoprofen concentration analysis

Fifty milligrams of tissues were homogenized in methanol. Ketoprofen in the homogenates and plasma (0.25 ml) was acidified and extracted by liquid–liquid extraction with diethyl ether. After evaporation of the organic phase, the residue was dissolved in a methanol/water mixture and transferred into vials. Ketoprofen extracted from the plasma and tissues was assayed by high-performance liquid chromatography with positive ion spray ionization tandem mass spectrometry detection (LC-MS-MS; 2695 separation module (Waters) and API-4000 (Applied Biosystems/MDS SCIEX).

Results and Discussion

Mexican hairless pigs are descendants of Iberian pigs. The name 'hairless' derives from its main

characteristic, namely, the absence of hair (or sparse hair) on the skin (Figure 1A).

A topical patch containing 20 mg of ketoprofen was applied to the back of Mexican hairless pigs for 24 h (Figure 1B). Blood was collected at 0, 1, 2, 4, 6, 8, 12, 24 (prior to patch removal) 28, 32 and 36 h after the patch application. Ketoprofen was first detected in the plasma at 8 h, and the concentration increased steadily up to 24 h (Figure 2). After removal of the ketoprofen patch, the plasma concentration decreased, but the drug could still be detected until the 36 h time-point.

Next, the ketoprofen concentrations in the tissue specimens were measured. Ketoprofen patches were applied on the back of Mexican hairless pigs for 12 h. Immediately after removal of the patch, the skin, subcutaneous fat, fascia and muscle at the center of the patch application site (Figure 3A) were excised en bloc (2 cm × 2 cm × 3 cm). The muscle was divided into superficial muscle up to 5 mm thickness and the remaining deep muscle. The highest ketoprofen concentration was obtained in the skin, followed by that in the subcutaneous fat, fascia, superficial muscle and deep muscle, in that order (Figure 3B). Interestingly, ketoprofen concentrations in the tissues decreased with increasing depth of the tissues, even though the composition of the tissues, tissue permeability to ketoprofen and the vascularity in the layers are totally different. The ketoprofen concentration in the deep muscle was the lowest among the tissues examined, but it was still higher than that in the plasma. There was no marked difference in ketoprofen concentrations in the

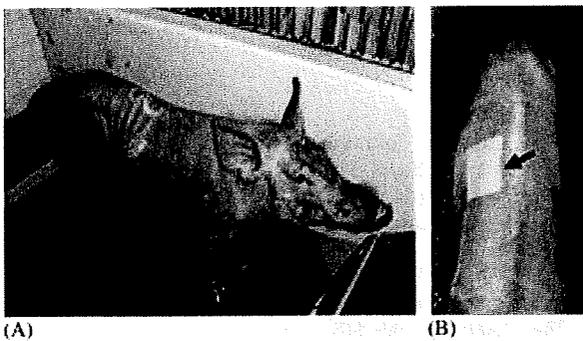(A)   (B)

Figure 1. Mexican hairless pig. (A) Typical appearance of a 12-month-old Mexican hairless pig. (B) Ketoprofen patch (arrow) applied on the back of the Mexican hairless pig
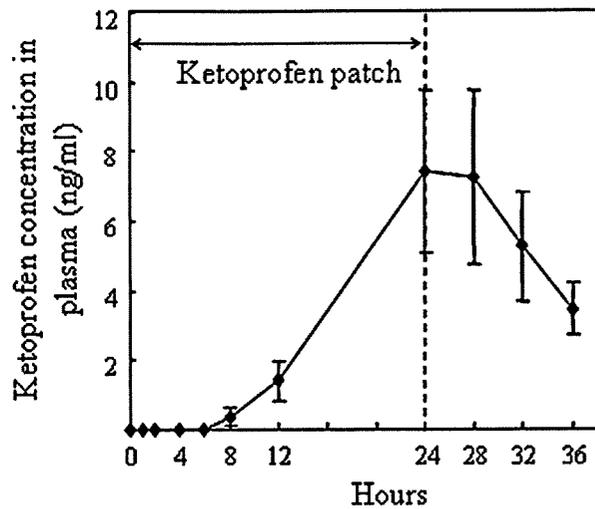
Figure 2. Ketoprofen concentrations in the plasma. Average values with SEM are shown ($n = 3$)
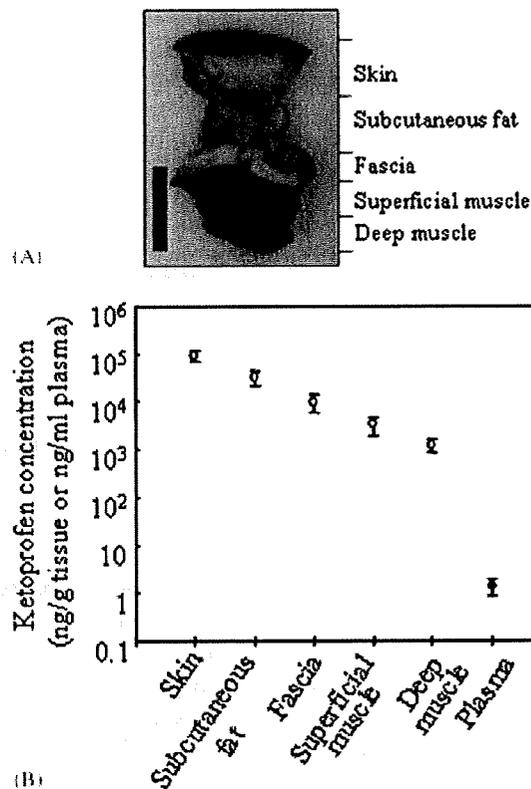
Figure 3. Ketoprofen concentration in the tissues. (A) Tissues from the center of the patch application site were excised en bloc and examined. Scale bar: 10 mm. (B) Ketoprofen concentration in each tissue at 12 h after the ketoprofen patch application. Average values with SEM are shown ($n = 4$)