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RESEARCH ARTICLE

In situ bioprinting for cartilage repair using a parallel manipulator

Supplementary File

(A) The cost of self-developing *in situ* bioprinter

The cost of self-developing *in situ* bioprinter is show in **Table S1**.

Table S1. List of the components and their corresponding costs

Component	Cost (RMB)
3D-printed extruder	80
Double shaft stepper motor	152
Aluminum-based cooling fin	1
Heat dissipation silicone sheet	10
Customized circuit	2
Light-emitting diode (LED)	22.5
Transparent acrylic sheet (0.5 mm thick)	3.2
Adjustable direct current regulated power supply	26
Stepper motors (×3)	120
Cartridge	8
Needle	1
Synchronous belt	10
Aluminum profile	500
Controller board	250
2004 LCD display with smart controller board	20
Customized stainless sheet	300
Total	1505.7

(B) Magnetic resonance imaging of knee joint

The magnetic resonance imaging (MRI) analysis was conducted in each rabbit at 12 weeks after surgery using a 3.0T MR scanner (GE750, USA) by using the following sequences (**Table S2**).

Table S2. MRI imaging sequences

Series description	Plane	Time of repetition	Time of echo	Field of view	Flip	Slice thickness	Distance factor	Matrix	Bandwidth
Fat-saturated T2-weighted TSE Imaging	Sagittal	2000 ms	72.0 ms	70 mm	150°	2 mm	10%	256 × 256	199 Hz/pixel
PD-weighted TSE imaging	Sagittal	2000 ms	36.0 ms	70 mm	150°	2 mm	10%	256 × 256	199 Hz/pixel
3D T1-weighted spoiled GRE imaging	Sagittal	26 ms	4.6 ms	60 mm	25°	1 mm	20%	512 × 256	200 Hz/pixel

(C) Gross observation

The distal part of the femurs was harvested and photographed. Quantitative evaluation of cartilage repair was performed using the International Cartilage Repair Society (ICRS) scoring system (**Table S3**) based on the gross observation.[1]

Table 3. ICRS macroscopic evaluation of cartilage repair

ICRS assessment of cartilage repair	Score
Degree of defect repair	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border <1 mm	3
3/4 of graft integrated, 1/4 with a notable border >1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	1
From no contact to 1/4 of graft integrated with surrounding cartilage	0
Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracks	2
Several, small or few but large fissures	1
Total degeneration of a grafted area	0
Overall repair assessment	
Grade I: normal	3
Grade II: nearly normal	2
Grade III: abnormal	1
Grade IV: severely abnormal	0

(D) Histological assessment

The repaired knees were harvested in 12 weeks after surgery and subsequently fixed in 10% formaldehyde for 3 days at room temperature. The fixed samples were decalcified in a mixture composing of formic acid, neutral buffered formaldehyde (40%), and phosphate-buffered saline in a volume fraction of 1:1:8. After decalcification for a week, the samples were trimmed, dehydrated, and embedded in paraffin. Sections with 5 μm thickness each were cut from the repair site and then stained with hematoxylin and eosin (H&E), Safranin O-fast green (SO&FG), Toluidine blue (TB) staining, and immunostaining of type II collagen antibody (1:300 dilution) according to manufacturer's protocols.

According to the Wakitani histological scoring system, the therapeutic efficacy of the cartilage defects can be quantitatively evaluated. The morphology of the cell and cartilage matrix can be determined by the TB staining and the SO&FG staining (**Table S4**).[2]

Table S4. Histological scoring system for evaluation of repair of cartilage defects

(A) Features of repaired cartilage	Score
Cell morphology	
Hyaline cartilage	6
Mostly hyaline cartilage: >3/4	5
Partly hyaline cartilage: 1/4–3/4	4
Mostly fibro-cartilage: >3/4	3
Partly fibro-cartilage: 1/4–3/4	2
Mostly non-cartilage	1
None cartilage only	0
Matrix staining (metachromasia)	
Normal (compared to host)	4
Slightly reduced staining: >3/4	3
Moderately reduced staining: 1/4–3/4	2
Remarkably reduced staining: <1/4	1
No metachromatic staining	0
Surface regularity	
Smooth: >3/4	3
Moderate: 1/2–3/4	2
Irregular: 1/4–1/2	1
Severe irregular	0
Thickness of the defect	
Normal: > 2/3	2
Moderate: 1/3–2/3	1
Thin: <1/3	0
Integration of repaired tissue to the surrounding articular cartilage	
Both edges integrated	2
One edge integrated	1
Both edges not integrated	0
Arrangement of repair cartilage	
Column-like arrangement	2
Partly column-like arrangement	1
Disordered	0
(B) Features of surrounding tissue	**Score**
Remodeling of subchondral bone	
Complete reconstruction	3
Continuous but incomplete reconstruction	2
Discontinuous, greater than 50% reconstruction	1
Discontinuous, less than 50% reconstruction	
Effect on adjacent cartilage	
Normal (compared to host)	3
Slightly reduced staining: >3/4	2
Remarkably reduced staining: 1/4–3/4	1
Little or no metachromatic staining: <1/4	0

References

1. van den Borne MP, Raijmakers NJ, Vanlauwe J, et al. International Cartilage Repair Society (ICRS) and Oswestry macroscopic cartilage evaluation scores validated for use in autologous chondrocyte implantation (ACI) and microfracture. *Osteoarthr Cartil*. 2007;15(12):1397-1402.
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2. Kawaguchi A, Nakaya H, Okabe T, et al. Blocking of tumor necrosis factor activity promotes natural repair of osteochondral defects in rabbit knee. *Acta Orthop*. 2009;80(5):606-611.
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