Bone regeneration using uncultured cells of bone marrow aspirate concentrate

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【Objectives】

Regenerative therapy with cultured bone marrow MSCs is associated with uncertainties with regard to the extent of bone regeneration. This technique is expensive and complex. In this study, we examined the bone-inducing ability of uncultured cells of bone marrow aspirate concentrate (BMAC).

Bone regenerative therapy

Cultured cell therapy: Complex / Safety? / Expensive / Uncertainty

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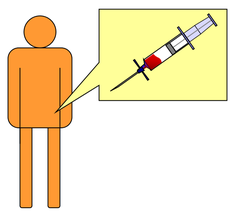
Bone Marrow Aspirate (BMA): Easy / Safe / Costeffective



Can we regenerate bone using BMA without culture procedure?

Clinical application to bone regeneration using BMA

- Orthopedic surgery
  (Carter JD 2009, Ploumis A 2010, Yamada T 2011)
- Oral maxillofacial surgery
  (Rickert D 2011)

→ BMA promotes bone formation → BMA concentrate (BMAC)?

- Isolation and concentration of mononuclear cells
- Growth factors
- Fibrin network

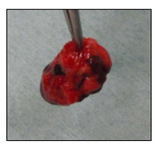
【Methods】

Experiment1 *ectopic bone formation model*

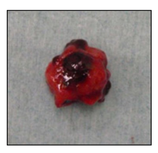
Animals: Four adult beagle dogs (male, approximately 10 kg)

Preparation of implant materials

- **BMAC group**: **β-tricalcium phosphate (β-TCP) with BMAC**
  anti-coagulant CPD2ml＋BMA13ml＋β-TCP
  centrifuge (2500G 15min)



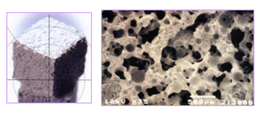
- **BMA group**: **β-TCP with nonconcentrated BMA**
  anti-coagulant CPD2ml＋BMA2ml＋β-TCP
  2%$CaCl_2$ added to form coagulation.



- **TCP group**: **β-TCP alone**

β-TCP was implanted into the back muscle of dogs.

Porous β-TCP blocks, 5×5×5mm, porous size：200–400 μm
(Osferion, Olympus Terumo Biomaterials Co, Japan)



Bone marrow is extracted from the iliac and femur bone.

Cell counts of BMA and BMAC

BMA / BMAC → 1w culture → DMEM+10%FBS → Cell counter

We compared the number of bone marrow cells that could be cultured for 1 week and collected between the BMAC group and BMA group.

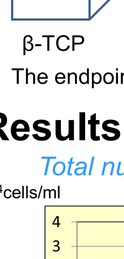
Platelet counts･Fibrinogen and TGF-β concentrations

Flow cytometrical analysis Characterization of mononuclear cells were analyzed by FACS.

Morphometric analysis by Scanning electron microscopy (SEM)

Ability to induce bone formation at 3 and 6 weeks after surgery （N=8）

Histological evaluation： Decalcified specimens (HE stain)



β-TCP

Selection two levels of sections per sample
2 specimens sliced at the quarter and middle section

Quantitation of bone formation area

We compared the ability to induce bone formation between the three groups at 3 and 6 weeks after surgery.

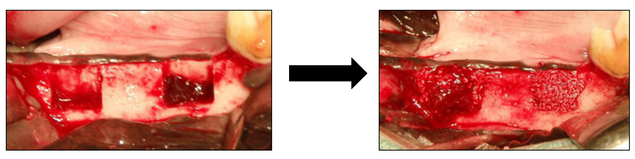
Histological evaluation was performed in decalcified specimens (HE stain) at two levels of sections per sample.

The endpoint differences between the groups were analyzed using t-test ($p < 0.05$).

Experiment2

We evaluated the bone-inducing ability in bone defects (8×7×4mm) of canine mandible.

Animals: Twelve adult beagle dogs (male, approximately10 kg)



Initially, all premolars in the mandible (P1–P4) were removed to create edentulous ridges. Two bone defects (length 8mm, height 7mm, depth 4mm) were created on each side of the mandible and the buccal bone plate was removed.

- **BMA group**： β-TCP 0.125g + Bone marrow aspirate 3ml
- **TCP group**： β-TCP 0.125g alone

These materials were filled into each bone defects of mandible.

porous**β-TCP**
(Osferion®, Olympus Terumo Biomaterials Co, Japan)

particle size: 0.5–1.5 mm,
porosity of 75%,
pore size:100–400 μm

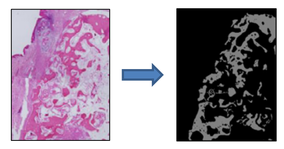
Results were evaluated at 6 and 12 weeks after surgery (N=6).

Radiographical analysis (micro-CT)

Measurements were performed for five areas of each grafted site.
The ROI was set as the size of 7×4 mm. The regenerated area (area of the new bone and β-TCP) of the ROI part was measured.

Histological analysis

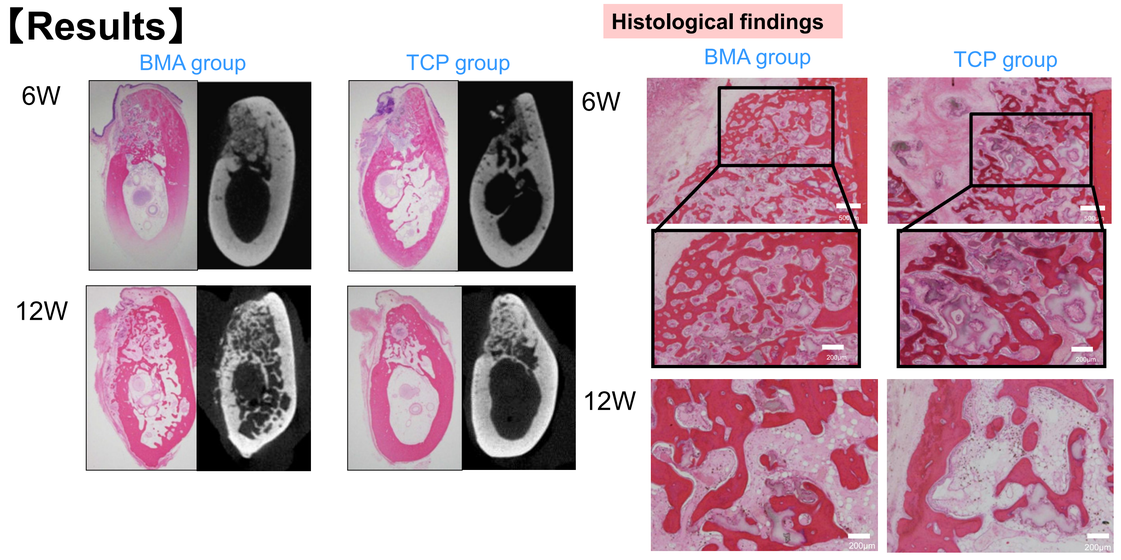
Decalcified tissue specimens (HE stain) from each defect were analyzed histologically.
The area of newly formed bone of each specimen were measured and analyzed.
The ROI was set as the size of 7×4 mm.
The area of the new bone of the ROI part was measured.



The endpoint differences between the groups were analyzed using the Mann–Whitney U test ($p < 0.05$).

【Results】

Histological findings



The regenerated area (area of the new bone and β-TCP)(pixel)

		average	*P* value	
6w	BMA group	6359 (1546)	0.032	*
	TCP group	5793 (1566)		
12w	BMA group	4842 (1372)	0.023	*
	TCP group	3959(1054)		

New bone area (mm^2)

6w	BMA group	6.15 (2.98)	0.02	*
	TCP group	4.32 (1.13)		
12w	BMA group	6.83 (2.60)	0.57	
	TCP group	6.41(3.09)		

* *P<0.05*

In the TCP group, many residual TCP was noted, and the crest width was not sufficiently augmented and the outline of the buccal alveolar plate was depressed with epithelial invagination. The bone trabecula and bone marrow were still immature in both group. In the BMA group, the contour of the alveolar bone crest was well retained.
A significant difference was observed between the control group and experimental groups (P<0.05) at 6 and 12 weeks.

Platelet counts･Fibrinogen concentrations of BMAC

	Average	Normal range	（N=4）
Platelet counts	15.8±0.5	20～50	(×10^4/μl)
Fibrinogen concentrations	361.75±27.6	200～400	(mg/d l)

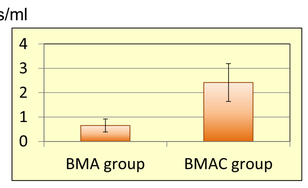
The average platelet counts in BMAC was slightly-decreased than the normal range. The average fibrinogen concentrations in BMAC remain within the normal range.

The concentrations of TGF-β1 （N=7）

The concentrations of TGF-β1 were undetectable or lower than 62.5 pg/ml.
TGF-β1 is platelet derived growth factor. Because, the concentrations of TGF-β1 were linked to platelet counts.

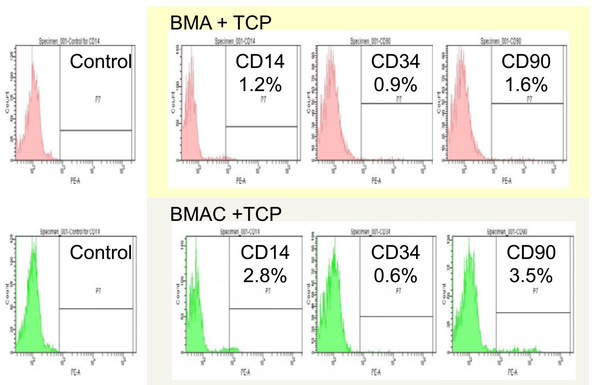
Total number of attached cells

×10^4cells/ml

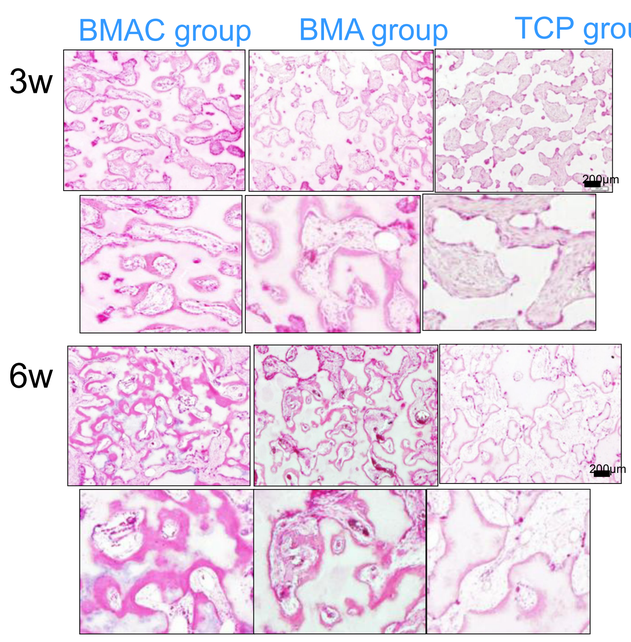


The number of bone marrow attached cells from the BMAC group was 4.9-fold enhancement of the number from the BMA group.

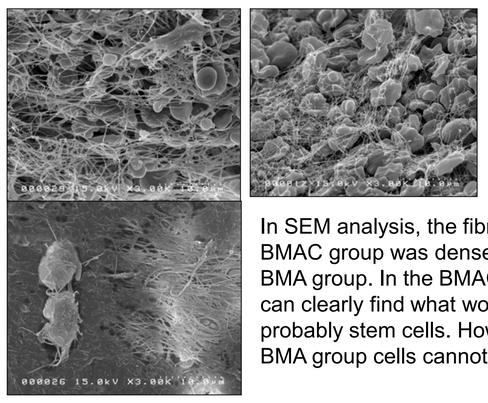
Characterization of MSCs



Total number: 6.2-9.8 ×10^6 cells



BMAC / BMA



In SEM analysis, the fibrin network of BMAC group was dense than that of BMA group. In the BMAC group we can clearly find what would be probably stem cells. However in the BMA group cells cannot be detected.

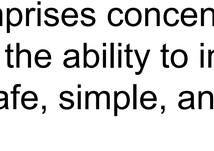
New bone area (mm^2)

		average	*P* value	
3w	BMAC group	0.79	0.081	0.033
	BMA group	0.06	0.231	
	TCP group	0.00		
6w	BMAC group	1.96	0.322	0.013
	BMA group	1.52	0.185	
	TCP group	0.61		

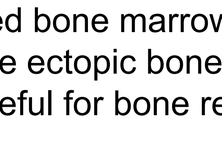
Ability of bone formation was significantly higher in the BMAC group than in the TCP group at both 3 and 6 weeks. In the BMA group, ability of bone formation was higher than the TCP groups. However bone growth in the BMAC group was faster than the BMA group. TCP groups show no new bone formation at 3 weeks and slight bone formation at 6 weeks. In the BMAC and BMA group, and in the BMA and TCP group, there were no significant difference between each group.

【Discussion】

3w BMAC group
MSCs: 3.4×10^5 cells



4w Cultured MSCs
2×10^6 cells



In another experiment, β-TCP block with cultured MSCs was implanted into the back muscle of dogs using the same method.
The numbers of cells in BMAC was much less than that of the cultured cells. But the results of BMAC group were better than the cultured one. Therefore, we speculate that the number of MSCs was not the most important factor for bone formation.

Why does BMA promote bone formation?

- Increase of cells from BMA
- Fibrin network (as scaffold)
- Effect of autogenous Extra cellar Matrix (ECM)?
- Effect of growth factors?

Three important factors of regeneration therapy are signal, cells and scaffold. BMA includes bone marrow stromal cells and MSCs as cells, fibrin as scaffold, growth factors from BMA or platelets as signal. We think that of signal and scaffold factors are more important than cells.

Future plan

Effect of autogenous extra cellar matrix and growth factors were not clear.
The optimal type of cells, ECM and growth factors for bone formation in BMA should be investigated.

【Conclusion】

In this study, increase of cells from BMA and the importance of fibrin network as scaffold were indicated. These findings indicate that BMAC, which comprises concentrated bone marrow stem cells and a fibrin scaffold that has the ability to induce ectopic bone formation; furthermore, this technique is safe, simple, and useful for bone regeneration in the clinical setting.