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About OMICS Group Conferences


OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

NIMS 

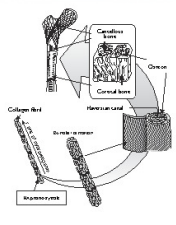
Bone-like Nanocomposite of Hydroxyapatite and Collagen as Artificial Bone

Masanori Kikuchi
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NIMS 

Background


Bone has a hierarchic structure from nano- to macro-scale. The primary structure is that hydroxyapatite nanocrystals are regularly aligned along collagen fibers. The material which has similar nanostructure and chemical composition will be better artificial bone.



The diagram illustrates the hierarchical structure of bone. It shows 'Nanocrystals' at the base, which form 'Collagen fibers'. These fibers are organized into 'Fibrils', which then form 'Fibers'. The fibers are further organized into 'Fibrillar bundles', which form 'Osteons'. The osteons are arranged in a 'Cortical bone' structure, which is part of the overall 'Bone' structure.

NIMS 


Hypothesis

NIMS 

Hypothesis


The sizes of HAp nanocrystals and collagen molecules are too small for cells to manipulate directly. Thus, the nanostructure of bone would be formed by a self-organization mechanism via interfacial interaction between HAp and collagen.

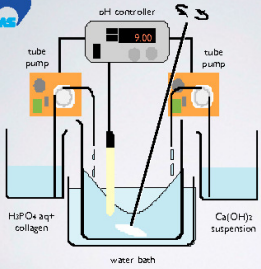
That is, the nanostructure of bone can be reproduced by mimic of cell roles in bone formation, i.e., control of physicochemical conditions.

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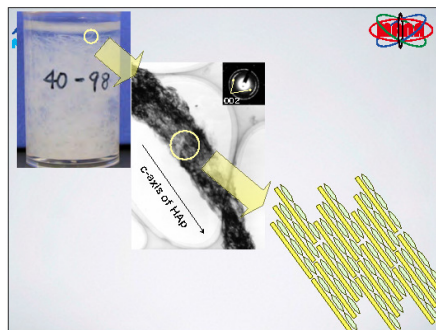
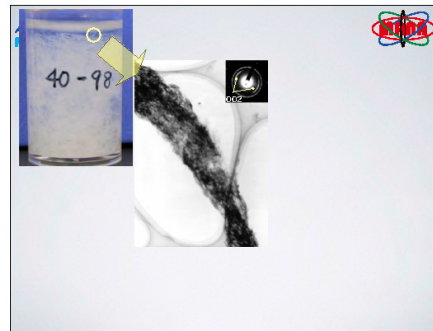
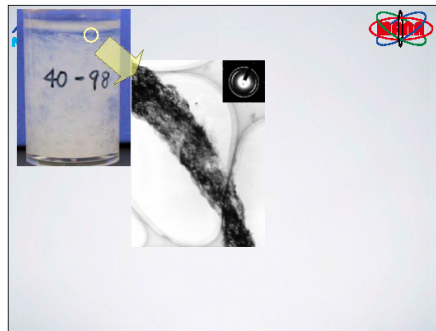
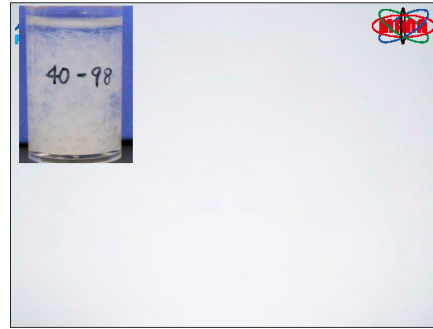
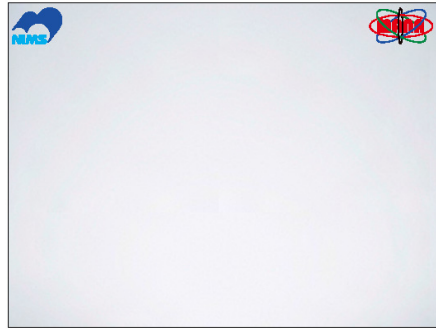
Hypothesis

Reproduction of bone-like nanostructure and chemical composition using self-organization of hydroxyapatite and collagen under biomimetic condition.

NIMS 

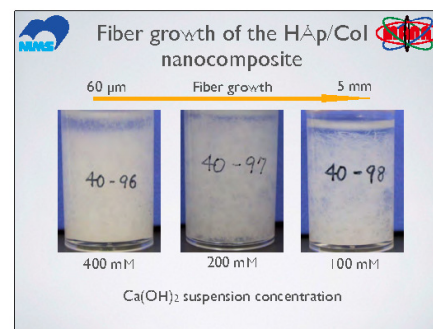
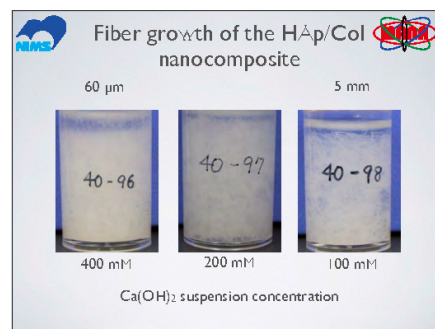
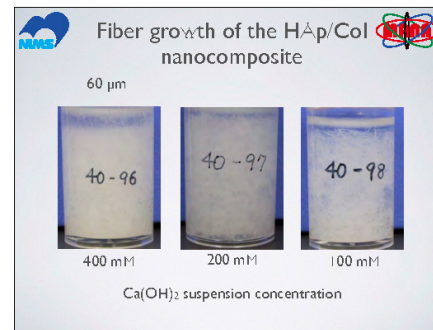
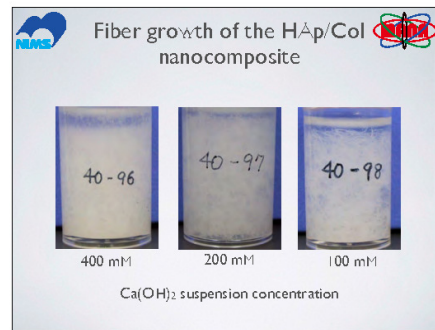
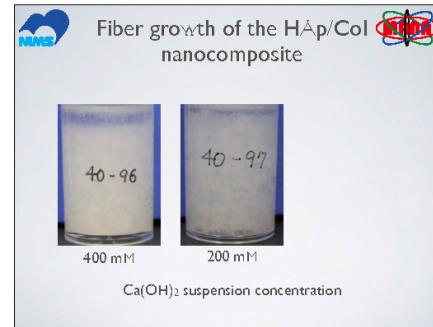
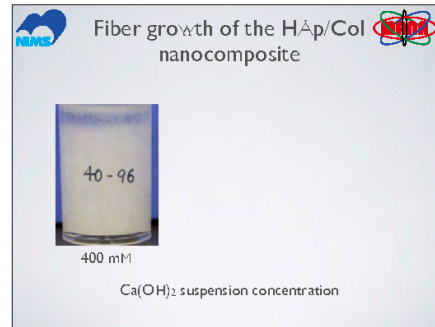


The diagram shows a 'simultaneous titration apparatus' used for the self-organization of HAp and collagen. It consists of a central 'water bath' containing a beaker. Two 'tube pumps' are connected to the bath: one for 'H₂PO₄ aq + collagen' and another for 'Ca(OH)₂ suspension'. An 'pH controller' is connected to the bath, with a digital display showing '6.00'. The text states: 'We prepared the "simultaneous titration apparatus" for encouraging of biomimetic self-organization process of HAp and collagen.'



Fiber growth of the HAp/Col nanocomposite

Ca(OH)₂ suspension concentration



Fiber growth of the HAp/Col nanocomposite

60 μ m Fiber growth 5 mm

We can synthesis large amount of bone-like nanocomposite via the self-organization process with controlled length and HAp/Col mass ratio.

400 mM 200 mM 100 mM

Ca(OH)₂ suspension concentration

Regeneration of segmental bone defect on beagle's tibia

Surgical operation

Material

3 Months after Surgery

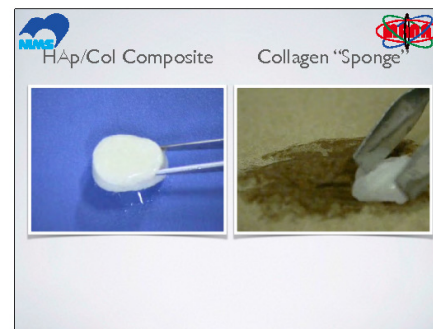
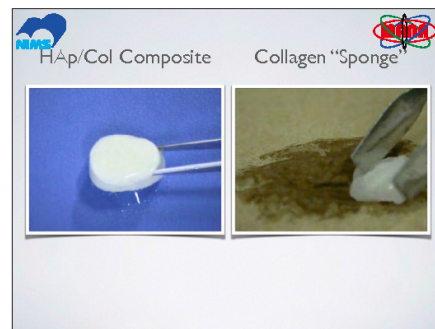
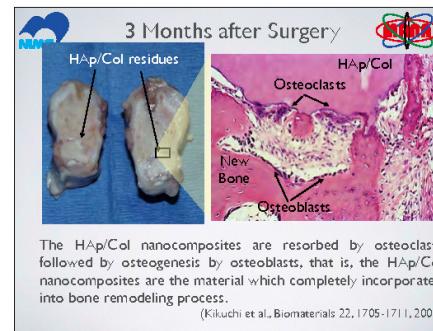
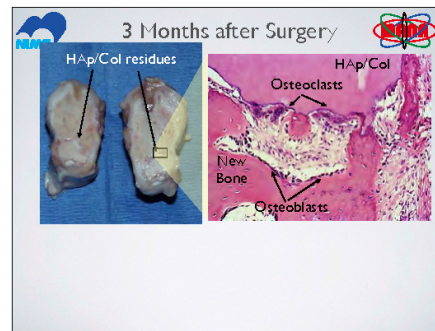
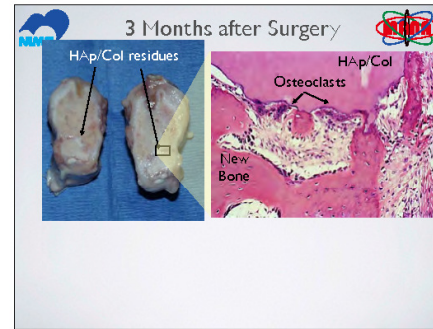
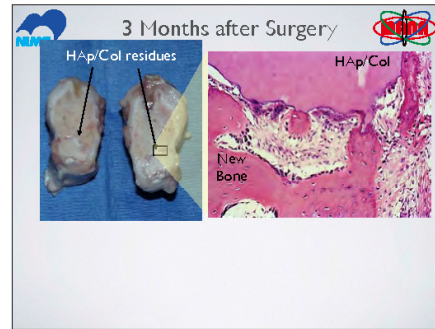
3 Months after Surgery



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Original size of the HAp/Col

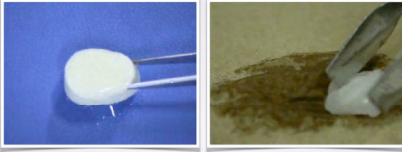
3 Months after Surgery

HAp/Col residues







HAp/Col Composite Collagen "Sponge"



The HAp/Col sponge is available in Japan as Refit® from April, 2013.






Clinical Test



Clinical Test

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- Clinical test for patients was performed in following 7 hospitals:
 - Tokyo Medical and Dental University Hospital
 - Tsuchura Hospital
 - Keio University Hospital
 - Cancer Research Institute Ariake Hospital
 - Kyoto University Hospital
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- Control material for the HAp/Col was commercially available porous β -tricalcium phosphate (β -TCP) bone filler, Osferion® of Olympus Terumo Biomaterials. (Requested by Pharmaceuticals and Medical Devices Agency (PMDA), Japan.)






Clinical Test



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- Fill to bone defect at donor site of autograft.



Comparison of Remarkable Efficiency at 24 Weeks after Operation

Group	Amount filled in	Number of patients	Number of remarkable efficiency	Remarkable efficiency rate
HAp/Co	0 < X < 3	38	26	68.4
TCP		38	22	57.9
HAp/Co	3 ≤ X < 10	8	0	55.6
TCP		8	6	33.3
HAp/Co	10 ≤ X ≤ 30	7	5	71.4
TCP		7	0	0
HAp/Co	Total	63	4	65
TCP		63	28	44.4

Manhnel-Hanszel test showed difference between groups, 20.6%, was significant. In addition, HAp/Co efficiency was better than TCP (Osterion®) after 4 weeks operation.





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- We speculated that these inflammations were “good” biological reactions that tissues prepared to turn into regeneration process. (In fact, with anti-inflammatory agents, remodeling process stops.)




HAp/Col Coating on Titanium for Subperiosteum Anchorage Device for Orthodontics




Animal test


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- Animal
 - ✓ 12 week-old male SD-rat
 - ✓ body weight of 360-370 g




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 - ✓ Pure titanium wires 0.5 mm in diameter and 12 mm in length
 - ✓ HAp/Col dip-coated
 - ✓ HAp biomimetic coated
 - ✓ bare




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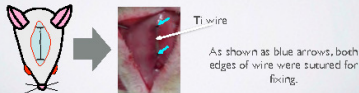
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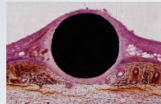


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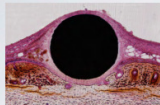


Histology

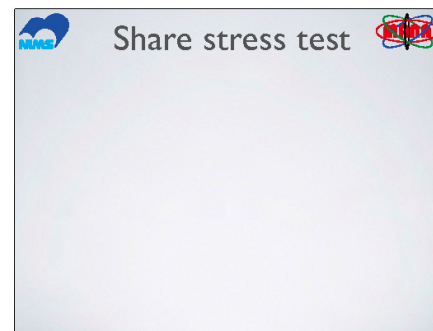
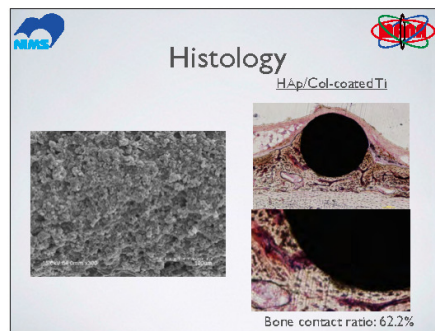
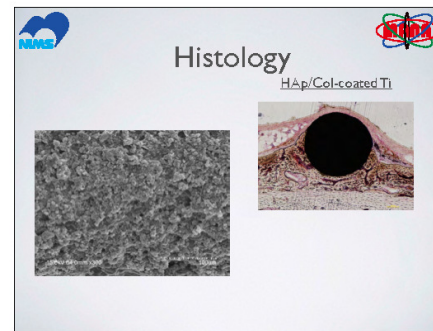
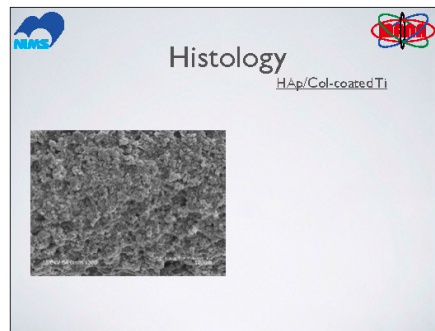
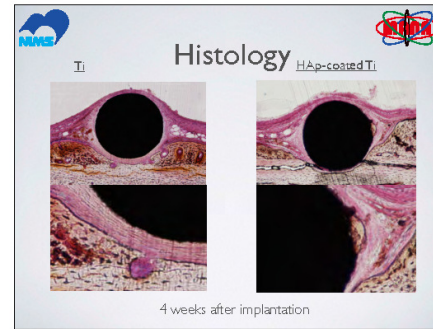
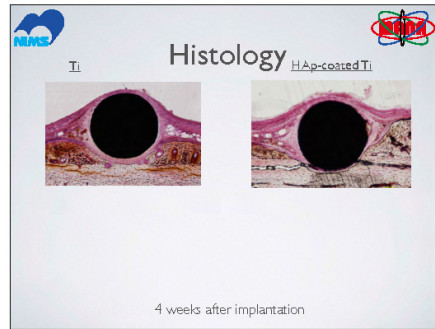


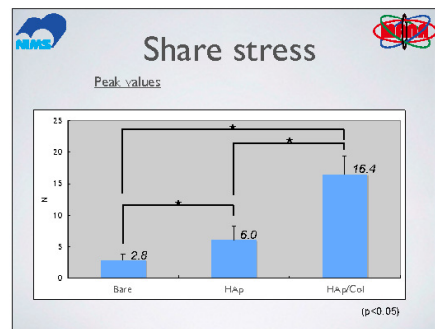
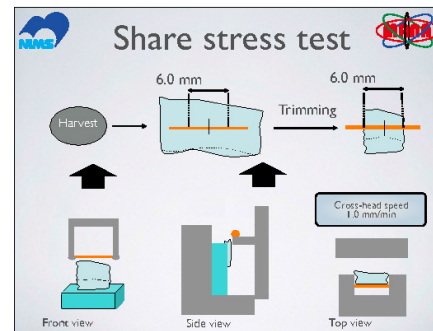
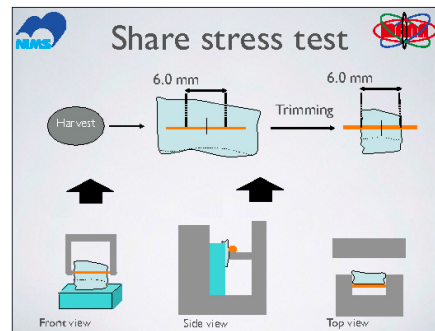
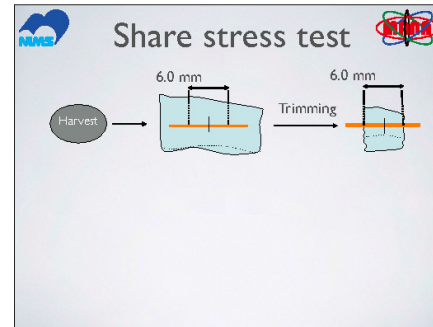
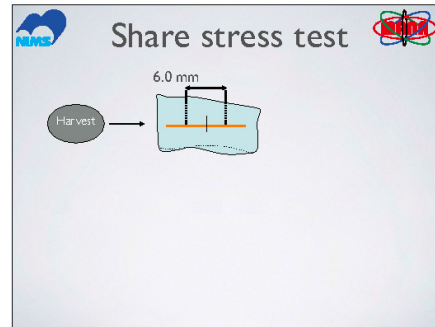
4 weeks after implantation

Histology





4 weeks after implantation







Injectable HAp/Col
 HAp/Col + Na-Alginate
 90 : 10 in mass






Preparation of the HAp/Col Powder

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Compacting by squeezing water in specially designed mold for uniaxial pressing






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Freeze-drying

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Crushing by hand followed by ball-milling

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Classified by a sieving to collect 100 – 212 μm in diameter

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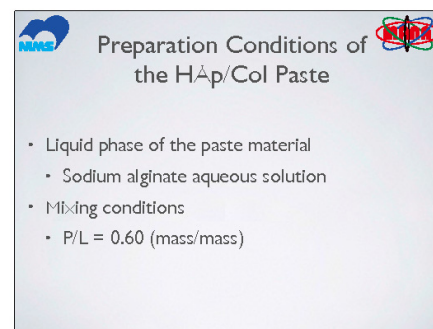
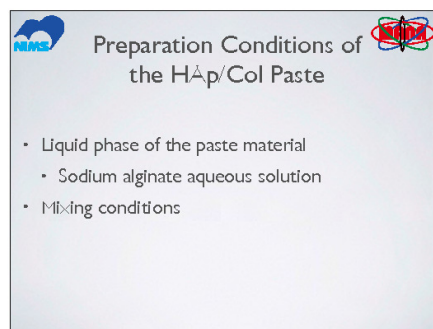
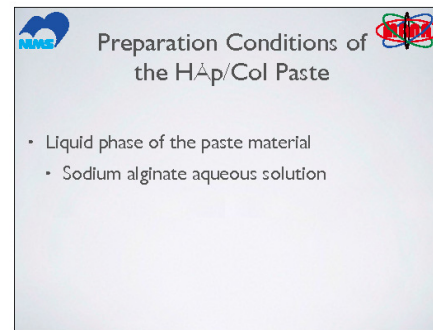
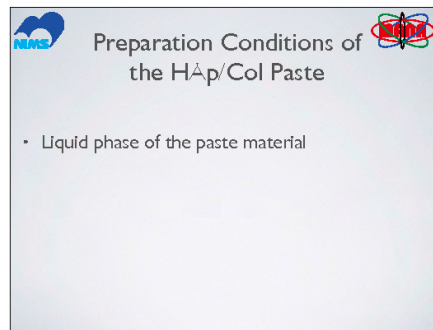
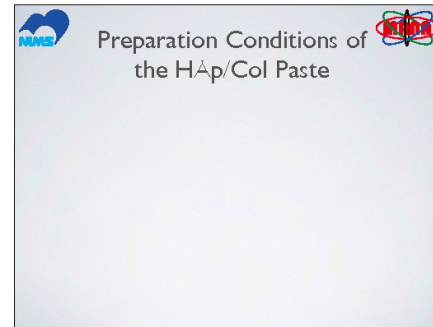
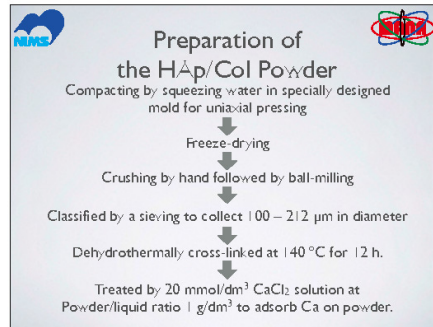
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Dehydrothermally cross-linked at 140 °C for 12 h.



Preparation Conditions of the HAp/Col Paste

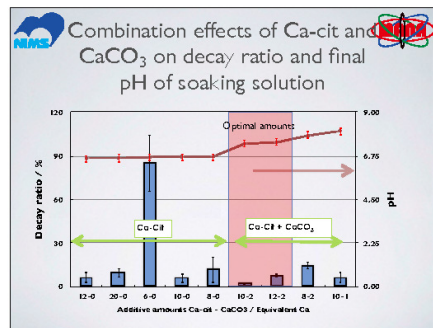
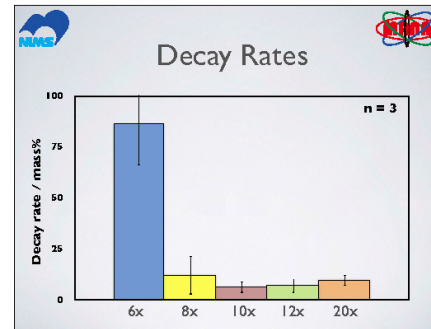
- Liquid phase of the paste material
 - Sodium alginate aqueous solution
- Mixing conditions
 - P/L = 0.60 (mass/mass)
 - HAp/Col : Na-Alg = 9 : 1 (mass:mass)

Decay Property Test

Ca-Cit Amounts	6	8	10	12	20
Final pH of PBS	6.70 ± 0.02	6.72 ± 0.3	6.72 ± 0.06	6.63 ± 0.05	6.64 ± 0.6

Photographs of Paste after Test

Photograph of the Paste (12x Ca-Cit)



Hardening in CaCl₂ Solution for Rapid Prototyping Use

HAp/Col-Alg paste injected into 100 mM CaCl₂ solution immediately hardened.

  **Hardening in CaCl₂ Solution for Rapid Prototyping Use**

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 Hardened paste collected from CaCl₂ solution remained its shape.



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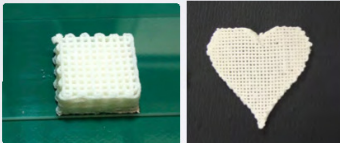
 Hardened paste collected from CaCl₂ solution remained its shape.

The paste had enough strength to handle.





  **Trial for Rapid Prototyping**

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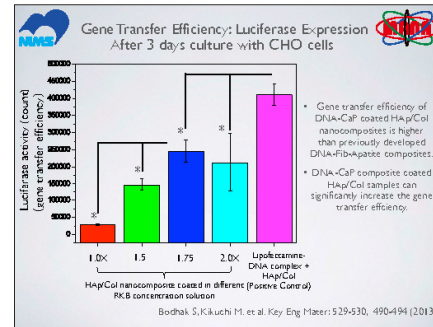
  **Trial for Rapid Prototyping**







Gene Transfer Bioresorbable Substrate

Collaborate with National Institute of Advanced Industrial Science and Technology








Conclusion



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 - substituting it with new bone by bone remodeling process.






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- The nanostructure of the HAp/Col allowed
 - enhancing osteoblastic activities.
 - supporting osteoclastic differentiation through osteoblasts
 - substituting it with new bone by bone remodeling process.
- Biomimetic nanostructure with controlled multi-scale structure can allow cell-free regeneration of large bone defect, even better than to β -TCP in the human clinical trial.






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- HAp/Col can be utilized for injectable artificial bone and rapid-prototyped (fine structure controlled) artificial bone by mixing with sodium alginate or other hydrogel formable organic substances, such as collagen.
- HAp/Col could be used for area specific gene transferable scaffold in vivo as well as in vitro with comparatively higher and/or safer transfection efficiency in comparison to present viral or lipid transfection.



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