PRODUCT HANDBOOK

A Revolution in Biomaterials
Synthetic Bone Graft Substitute

Prepared by:

GranuLab (M) Sdn. Bhd.(693189-U)
www.granulab.com
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SUMMARY

The number of patients suffering from disease or injury that leads to bone damage and loss are on the increase. In orthopaedic surgery bone grafting is an important part of the surgeon’s armament. Bone available from the same patient (autogenous) or from donors (allogenic) and animals (xenogenous) are limited and are not without various problems. Therefore, synthetic materials have great potential for use as a bone graft substitute, especially hydroxyapatite (HA), which has a similar chemical composition to the mineral phase of bone. IIUM, AMREC-SIRIM, UKM and USM have collaborated in an Intensified Research in Priority Areas (IRPA) research project (No. 03-01-03-0000-PR0026/05) to develop such a material. This work represents the first product developed by the group that is being commercialised.

AMREC-SIRIM has developed a novel method for the fabrication of HA using solid state fusion (Malaysian Patent Pending PI 2004 0748), which produces HA in a shorter time period with good reproducibility. HA powder and granules produced by this method was fully characterized and shown to fulfil the criteria for the American Society for Testing and Materials (ASTM) F1185-88 (1993) Standards, which is the “Standard Specification for Composition of Ceramic Hydroxyapatite for Surgical Implants”. The materials and product was also put through a series of in vitro and in vivo biocompatibility evaluation studies by IIUM, UKM and USM. The studies were done using the FDA and ISO matrices as a guide, including the ISO 10993 Standards for In Vitro and In Vivo Cytotoxicology & Biocompatibility Tests. The results of the studies shows that the materials and product is fully biocompatible for human use and they are strongly bioactive and osteoconductive. The product was named GranuMaS™ in 2005.

DESCRIPTION

GranuMaS™ is a granular synthetic bone graft material that comes in 4 different size range; 200µm -500µm, 500µm -1000µm, 1000µm -2500µm and 2500µm -5000µm. It is a pure hydroxyapatite (HA) material with a chemical formula of Ca_{10}(PO_4)_6(OH)_2. It is an excellent alternative material for the repair of bone defects due to its chemical composition being similar to human bone material. GranuMaS™ can be used in surgeries requiring bone grafting for non-loading bearing applications such as in the Dental, ENT, Orthopaedics and Maxillofacial specialties. GranuMaS™ was invented in AMREC, SIRIM Berhad using a patented process [Malaysian Patent Pending P120040748] and have passed all of the required criteria for ASTM F1185-88 (1993) Standard for Composition of Hydroxyapatite (HA) for Surgical Implants. It is derived from pure commercial chemicals and Malaysian limestone. It has been successfully screened through various in vitro and in vivo tests and also clinical trials. Its highly osteoconductive properties promotes good callus formation and the subsequent healing of bone defects.
LITERATURE REVIEW SUMMARY

Bone graft surgery is a common procedure in surgical operations. Surgeons in the Orthopaedic, Dental, Maxillofacial, ENT and Neurosurgery do it almost on a daily basis. Presently there are a large number of materials available for the repair and reconstruction of skeletal defects\(^{(1)}\). Bone grafts are categorised into ‘autogenous’- bone implanted from one site to another within the same individual, ‘allogenous’– bone transferred from one individual to another from within the same species (man to man), ‘xenogenous’– bone from one species implanted to another species (animal to man) or synthetic materials\(^{(1,2)}\).

Autogenous bone graft is considered as the gold standard in today’s practice. However, only a limited quantity of autogenous bone graft is available for harvest from a patient at any one time\(^{(1)}\). There is also a significant rate of donor-site morbidity\(^{(3,4)}\). Up to 30% of patients who undergone a procedure requiring bone grafts have some complications at the donor site, which includes chronic pain, infection or anatomical defects. On the other hand, one of the main concerns with allogenous bone and tissues is the transmission of infection, especially Hepatitis B and C, and HIV infections\(^{(5)}\). Despite all of this, and amid concerns about its safety, the use of allogenous bone graft for skeletal restoration has been generally accepted and will likely to continue until alternative methods are found\(^{(6)}\). Use of xenogenous bone grafts has also not been very popular because of the similar medical and cultural issues that are associated with allogenous bone graft\(^{(1,2)}\).

The most promising material for skeletal repair is therefore the synthetic material\(^{(2)}\). However, they are not readily available locally and are usually quite expensive. Collectively they are known as biomaterials and the most extensively investigated are the calcium phosphate bioceramics\(^{(7,8,9,10,11)}\). It has been shown to be non-toxic, non-inflammatory, biocompatible and osteoconductive in both animals and man. However, many of these products are still in the R&D stage. Two types of calcium phosphate bioceramics, specifically hydroxyapatite (HA) and tricalcium phosphate (TCP) has been extensively studied to be used in bone repair surgery. However, it is important to note that the biological apatite found in human bones is calcium-deficient and a carbonated apatite.

TCP is considered to be a resorbable biomaterial since it degrades relatively much faster in the biological environment than HA\(^{(12)}\). The β-crystalline form of TCP is more stable than the α-crystalline form and hence, it is β-TCP that is being widely commercialized in the last few years. The most commonly used commercially available calcium phosphate bioceramics bone graft substitute in Malaysia and the region is Chronos® developed by Synthes® in Switzerland, which is made from pure β-TCP. HA and β-TCP are very different materials with different properties and each are better suited for different surgical applications. There is separate American Society for Testing and Materials (ASTM) standards available for β-TCP and HA that are to be used in bone repair and augmentation surgery.

The most promising material for skeletal repair is therefore the synthetic material\(^{(2)}\). However, they are not readily available locally and are usually quite expensive.
Collectively they are known as biomaterials and the most extensively investigated are the calcium phosphate bioceramics\(^{7,8,9,10,11}\). It has been shown to be non-toxic, non-inflammatory, biocompatible and osteoconductive in both animals and man. However, most of these products are still in the R&D stage.

In Malaysia, there are some on-going R&D activities in this field, which is currently fragmented with the absence of integrated efforts among the various disciplines. This is because the study of biomaterials requires a multidisciplinary approach in which both the research scientist and the practicing clinician have to work together to integrate the biological, material and engineering sciences\(^{12}\). This study represents the first attempt by the authors and their institutions to work together on developing a synthetic bone graft substitute based on HA.

References

**PRODUCTION OF GranuMaSTM**

The production of GranuMaSTM is done via wet synthesis and a novel solid state reaction method. The solid state reaction process is patented (Malaysian Patent Pending PI20040748). The aqueous precipitate formed is then spray dried and sintered at ~1000°C to form the granular HA. Different sized granules can be produced using this method according to the end-user needs. A summary of the process pathway is shown in the flowchart below.

**Processing Methods of GranuMaS**

```
Wet Synthesis / SSR
  ↓
Aqueous precipitate
  ↓
Spray Drying
  ↓
Firing (T~ 1000°C)
  ↓
Granular HA
```
The spray drying machine used in the making of GranuMaSTM

GranuMaSTM is available in different granule sizes.

(a) (200 - 500 μm)  (b) (500 - 1000 μm)  (c) (1000 - 2500 μm)  (d) (2500 - 5000 μm)
QUALITY CONTROL

The production of GranuMaSt™ is subjected to two (2) international standards to ensure that the quality of the finished product is of high quality and suitable for human implantation.

Firstly, the management of the production process is compliant to the ISO 9001:2000 standards. The raw materials have a complete chemical data sheet from the supplier and are fully characterised using X-ray diffraction (XRD), Inductive couple plasma (ICP), Scanning electron microscope (SEM), and Particle size analysis (PSA). All equipments used are calibrated on a regular basis.

Secondly, the American Society for Testing and Materials (ASTM) F1185-88 (1993) Standards, which is the “Standard Specification for Composition of Ceramic Hydroxyapatite for Surgical Implants”, was used to characterize the product. The four (4) criteria in this standard that are looked at to determine the suitability of HA to be used surgically in patients are:

Secondly, the American Society for Testing and Materials (ASTM) F1185-88 (1993) Standards, which is the “Standard Specification for Composition of Ceramic Hydroxyapatite for Surgical Implants”, was used to characterize the product. Four (4) criteria are looked at in this standard to determine the suitability of HA to be used surgically in patients. They are:

1. The quantitative X-ray diffraction analysis of the material must have a minimum HA content of ~95%.
2. The theoretical value for the calcium to phosphate (Ca/P) weight ratio should be 2.15 ± 0.11 for 95% purity.
3. The concentrations of heavy metals within the material should be below the Maximum Allowable Limit given.
4. All metals or oxides present within the material with a concentration of >0.1% must be listed.

HA Content
Using a 4-D XRD machine (as shown below), each batch of GranuMaSt™ produced is characterised fully and compared against the materials database available and also commercially available HA.
The XRD machine used for all characterisation of materials

XRD Results

The Quantitative X-ray Diffraction Analysis for GranuMaS™ shows that it consistently has a HA content of >99% for every batch produced. Thus, GranuMaS™ is comparable to commercial HA and is also compliant with the first ASTM F1185-88 (1993) Standards requirement.
**Ca/P Weight Ratio**

Experimental results using EDX on each batch of GranuMaSTM™ produced shows that the Ca/P weight ratio is within the theoretical value required by the ASTM F1185-88 (1993) as shown in the table below.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>ETHSWCS</th>
<th>GSWC1+2</th>
<th>GSWC3+4</th>
<th>GSWCS</th>
<th>GSWC6</th>
<th>GSWC7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (weight)</td>
<td>30.80± 0.05</td>
<td>42.50± 0.05</td>
<td>41.60± 0.05</td>
<td>41.70± 0.05</td>
<td>42.50± 0.05</td>
<td>41.90± 0.05</td>
</tr>
<tr>
<td>P (weight)</td>
<td>14.20± 0.05</td>
<td>19.10± 0.05</td>
<td>18.90± 0.05</td>
<td>18.90± 0.05</td>
<td>18.60± 0.05</td>
<td>18.30± 0.05</td>
</tr>
</tbody>
</table>

Ca/P weight ratio of GranuMasSTM batches

---

**Heavy Metal Content**

The concentration of the heavy metal content for the different batches of GranuMaSTM™ is measured using the inductive couple plasma method. The heavy metal content of GranuMaSTM™ was found to be well below the Maximum allowable Limit under the ASTM F1185-88 (1993) Standard as shown in the table below.

<table>
<thead>
<tr>
<th>Elements</th>
<th>ETHSWCS 5 (1993) Maximum Allowable Limit (ppm)</th>
<th>GSWC1+2 2 (± 0.20) detected (ppm)</th>
<th>ETHSWCS 5 (± 0.27) detected (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>5</td>
<td>0.35</td>
<td>0.34</td>
</tr>
<tr>
<td>Pb</td>
<td>30</td>
<td>2.40</td>
<td>3.30</td>
</tr>
<tr>
<td>As</td>
<td>3</td>
<td>1.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Hg</td>
<td>5</td>
<td>0.01</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Heavy metal content using ICP
Metal and Oxides List
The list of all metal and oxides found in the different batches of GranuMaS™ using ICP is shown below.

<table>
<thead>
<tr>
<th>Element</th>
<th>ETHS 1/VCS Detected (ppm)</th>
<th>OSW/C+2 Detected (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>96.6</td>
<td>123.8</td>
</tr>
<tr>
<td>Zn</td>
<td>0.24</td>
<td>2.0</td>
</tr>
<tr>
<td>K</td>
<td>115</td>
<td>107.1</td>
</tr>
<tr>
<td>Ni</td>
<td>0.53</td>
<td>0.73</td>
</tr>
<tr>
<td>Co</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>Na</td>
<td>272</td>
<td>&lt;1.8</td>
</tr>
<tr>
<td>Cr</td>
<td>6.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.02</td>
<td>-0.04</td>
</tr>
<tr>
<td>SB</td>
<td>0.17</td>
<td>0.24</td>
</tr>
<tr>
<td>Si</td>
<td>242</td>
<td>205.3</td>
</tr>
</tbody>
</table>

List of metals and oxides present in GranuMaS™

STERILISATION EFFECT STUDY

A comprehensive study was performed on GranuMaS™ by subjecting it to various sterilization methods. Samples of GranuMaS™ were triple-packed and sterilised using various methods; i.e. autoclave, dry heat, -irradiation or electron beam irradiation; for either one, two or three cycles with aging periods ranging from none to 3 months. Samples that underwent multiple cycles were either done so immediately or aged for 2 weeks in between each cycle. Samples were characterised using XRD before and after undergoing the sterilisation methods. The samples were also cultured for microbial load in nutrient agar and blood culture medium for up to 5 days at 37ºC.

The objectives of this study are:
1. To look at the bioburden of the product received
2. To investigate the effect the various sterilisation methods and protocols used have on the physical and chemical characteristics of GranuMaS™.
3. To make an objective decision on the optimal sterilisation method best used on GranuMaS™.

The study shows that the fabrication process produces material with no microbial contamination. The granules are able to withstand all 4 methods of sterilisation without any change to its chemical structure or phase composition. The best method for sterilising GranuMaS™ is with gamma-radiation at 25KGY for 3 hours 23 minutes and 54 seconds.
BIOCOMPATIBILITY EVALUATION

GranuMaSTM was subjected to a series of in vitro and in vivo biological evaluation tests as prescribed by the ISO 10993 and FDA matrices. Tests were run for both the synthesised material and also the final product. The scope of the evaluation includes cytotoxicity tests, genotoxicity tests and animal studies. The evaluation was performed at the Biocompatibility Lab in the National University Malaysia (UKM).

Cytotoxicity Test
The cytotoxicity tests evaluate the effect that the extract of the test samples have on the viability, proliferation, colony formation, cell cycle and apoptosis of cell cultures. Tests performed include the Neutral Red (NR) assay for cell viability using L929 fibroblast cells (up to 102% viability) and CRL-1427 osteoblast cells (107% viability); MTT (Tetrazolium Salt) assay for cell proliferation (up to 99.5% viability); V79 cell Colony Formation assay; Cell cycle evaluation using CRL-1543 osteoblast cells; apoptosis effect using Acridine Orange / Propidium Iodide (AO/PI) dual staining of V79 cells in DMEM with positive and negative test controls.
NR assay results for GranuMaSTM extract (top) in comparison with zinc sulphate heptahydrate (bottom) as the positive control.
MTT assay results for GranuMaST™ extract (top) in comparison with zinc sulphate heptahydrate (bottom) as the positive control.
Photomicrograph of L929 cells monolayer treated with GranuMaS™ extract (top) and with zinc sulphate heptahydrate as positive control (bottom)
Results for Colony Formation assay in V79 cell cultures of GranuMaSTM extract (purple line) when compared to the positive control using zinc sulphate heptahydrate (red line).

Unstained (left) and Giemsa stained (right) photomicrograph of the V79 cells colony formation assay for GranuMaSTM.
Evaluation of cell cycle using flow cytometric analysis of GranuMaS™ extract in CRL-1543 osteoblast cells

Normal looking L929 cells treated under fluorescence light microscopy after treated with extract of GranuMaS™ for 72 hours

Apoptotic bodies seen when L929 cells were treated with the positive control substance
Apoptotic effect of GranuMaS™ in L929 cells assessed by AO/PI Dual Staining method

Genotoxicity Tests
Genotoxicity tests were performed to evaluate the genetic toxicity of GranuMaS™ by studying the effects it has on bacterial reverse mutation, micronucleus formation, and cellular DNA damage. The studies performed include the Reverse Mutation assay using the Ames test; micronucleus assay in V79 cells; and the Alkaline Comet assay in L929 cells. Results showed that GranuMaS™ has no significant genotoxic effects towards the cells that it was tested on and is comparable with the negative controls.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/plate)</th>
<th>Number of revertant colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA1535</td>
<td>TA1537</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>396</td>
</tr>
<tr>
<td>HA extract</td>
<td>0.3125</td>
<td>638</td>
</tr>
<tr>
<td></td>
<td>0.625</td>
<td>572</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>716</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>117</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.005</td>
<td>122</td>
</tr>
</tbody>
</table>

Mutagenicity of GranuMaSTM extract against positive & negative controls in the Ames assay using *S. typhimurium* strains in the absence of S9 fraction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/plate</th>
<th>Number of revertant colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA1535</td>
<td>TA1537</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>356</td>
</tr>
<tr>
<td>HA extract</td>
<td>0.3125</td>
<td>397</td>
</tr>
<tr>
<td></td>
<td>0.625</td>
<td>425</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>380</td>
</tr>
<tr>
<td>2-aminoantracene</td>
<td>0.005-0.001</td>
<td>331</td>
</tr>
</tbody>
</table>

Mutagenicity of GranuMaSTM extract in the Ames assay using *S. typhimurium* strains in the presence of S9 fraction.
Results of *in vitro* Micronucleus Assay in V79 cells

V79 cells treated with extract of GranuMaS™ (Left), V79 cells showing presence of micronuclei (mitomycin C-treated) (Right).
Results of the Alkaline Comet Assay in L929 cells

L929 treated with GranuMaSTM extract shows no Comet sign (Left) compared to DNA strand breaks seen in positive control-treated L929 cells (Right).
Animal Studies
The animal studies performed include looking at the effect of irritation, sensitization and systemic toxicity of GranuMaS™ extract on New Zealand White (NZW) rabbits and guinea pigs. The studies done have shown that GranuMaS™ is not a primary irritant or toxic material.

<table>
<thead>
<tr>
<th>PSI Index</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 5.0</td>
<td>Not a primary irritant</td>
</tr>
<tr>
<td>Greater or equal to 5.0</td>
<td>Primary irritant</td>
</tr>
</tbody>
</table>

Primary Skin Irritation Classification - PSI Index

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Primary Skin Irritation Index (PSII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GranuMaS™</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Results of Primary Skin Irritation Assay (close-patched) with GranuMaS™ extract.

<table>
<thead>
<tr>
<th>Test article</th>
<th>Primary Irritation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl (0.9%)</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
</tr>
<tr>
<td>HA extract</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Results of Intracutaneous Irritation in rabbits treated with GranuMaS™.

<table>
<thead>
<tr>
<th>Tests article</th>
<th>% guinea pigs responding to an irritancy score of ≥ 1</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA extract (n=11)</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative control (n=5)</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive control (n=6)</td>
<td>80</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Results of Dermal Sensitization Assay (Buehler Method) in guinea pigs treated with GranuMaS™.

<table>
<thead>
<tr>
<th>Test article</th>
<th>Treatment</th>
<th>Biological Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl (0.9%)</td>
<td>Cottonseed Oil</td>
</tr>
<tr>
<td>HA extract</td>
<td>Extract</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

Results of Acute Systemic Toxicity Assay in mice treated with GranuMaS™
Primary Skin Irritation (PSI) of a rabbit treated with GranuMaS™ extract on abraded and non-abraded sites showing 0 score for erythema.

DNCP-treated guinea pig showing slight erythema in the Dermal Sensitisation Assay.
ANIMAL IMPLANTATION STUDIES

GranuMaSTM were surgically implanted in the proximal tibial metaphyses of New Zealand White rabbits and Merino sheep. The tibia were harvested after 2, 3, 4, 6 and 12 weeks. Undecalcified histology slides were produced from the specimens and evaluated under light microscopy using compound polarized light or Toulidine Blue, Masson Goldner’s Trichrome or von Kossa reagents. Comparison was also made at 12 weeks between other commercially available imported bone graft substitutes.

New bone formation is seen early, even at 2 weeks post-implantation, which further consolidates with significant mineralization at 3 weeks. The cortical defect made was bridged by well-mineralised new bone at 4 to 6 weeks. At 12 weeks GranuMaSTM is incorporated in the new bone formed across the defect created and also across the intramedullary cavity. GranuMaSTM has good potential for use in defects that requires cross-sectional strength such as fractures involving the subchondral bone. Presence of osteoclastic breakdown of GranuMaSTM was observed histologically.

Animal Holding facilities at IIUM, New Zealand White Rabbit are kept in a TecniplastSTM rabbit cage.

Animal operating theatre at IIUM. Kuantan.
Once the implanted bone has been harvested after designated period of time, it was then cut and undergoes a series of dehydration process and followed by a series infiltration process using Technovit 7200. The samples then were prepared to be sectioned to make thin histology slide.

A defect was made at the proximal tibiae. GranuMaST\textsuperscript{TM} inserted as bone filler

Sample mounted on perspex slides to form a sandwich for the cutting process.
The thin sectioned sample was then ground to the desired thickness and further polished for better quality.
The prepared slide undergoing staining process

An example of a cross section of a rabbit’s proximal tibiae produced using EXAKT system.

An example of a fractured NZW rabbit’s tibiae with a cortical autograft and metal implant in situ produced using the EXAKT system.
Toluidine Blue Stain

2 weeks post implantation (10x)

3 weeks post implantation (10x)

4 weeks post implantation (10x)

At 40x showing presence of a multinucleated giant cell (red arrow) against the surface of the implanted GranuMaS™
Masson Goldners Trichrome stain

2 weeks post implantation (10x) showing presence of well mineralised woven bone (bluish-green) and unmineralised bone (orange-red) between the GranuMaS™ granules (HA)

3 weeks of implantation (10x) showing presence of more new bone formation between the granules (HA)

Von Kossa Stain

Presence of collagen within the osteoid found between the granules at 2 weks. (10x)

Increased presence of collagen at 3 weeks. (10X)
Compound Polarized Light

2 weeks post implantation (2.5x)

3 weeks post implantation (2.5x)

4 weeks after implantation (2.5x)

6 weeks after implantation (2.5x)

12 weeks post GranuMaS™ implantation (2.5x) showing lamellar bone formed across the defect made and also across the intramedullary cavity, with incorporation of GranuMas™. This indicates high strength following healing, which not only contribute to return of the cortical strength but also cross-sectional strength.
Results of the Animal Implantation Study
New bone formation was seen early with significant mineralization at 3 weeks onwards. The cortical defect was bridged by well-mineralised new bone at 4 to 6 weeks and at 12 weeks GranuMaS™ has been fully incorporated in the new bone formed across the defect created and also across the intermedullary cavity. There is histological evidence of the osteoclastic breakdown of GranuMaS™.
GranuMaS™ IN DENTAL APPLICATION

The study was done in USM's School of Dental Sciences on healthy patients post tooth extraction according to GCP protocols with the informed consent of patients and regular follow-up. More than 70 patients have been included in this study to date. Clinically, no adverse reactions were seen, both locally and systematically. Prevention of alveolar ridge resorption post tooth extraction was also observed.

Methodology
Study design - Comparative cross sectional study

Population - Patients undergoing dental extraction in Hospital Universiti Sains Malaysia dental clinic

Inclusion Criteria - Age 18-48 years with mandibular molar & premolar teeth extraction

Exclusion criteria – Patients with systemic diseases, who are smokers or with poor oral hygiene

70 patients were recruited into a study and divided into the study group (n=32) and the control group n=38). The study group had GranuMaS™ inserted into their emptied tooth socket immediately after extraction of a tooth. While the control group did not have any material inserted in the emptied tooth socket after the tooth was extracted. The patients were followed up at 1 week and 6 months post tooth extraction. An Oral Panoramic radiograph (OPG) of the patient was taken and the bone height of the alveolar ridge was measured. Comparison of the measurements between the study group and control group were made and analysed statistically.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Follow up after 1 week</th>
<th>Follow up after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test material</td>
<td>32</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>38</td>
<td>34</td>
</tr>
</tbody>
</table>

Post-op follow up record

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Post 0p complication after 1 week n=32</th>
<th>Post op complication after 6 months n=28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test material</td>
<td>32</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Post-op complication reported
Extraction of a damaged tooth

Exposed tooth socket post tooth extraction

Insertion of GranuMaS™ in the extracted tooth socket
X-ray showing how the alveolar bone height is affected by an emptied tooth socket after healing

Bone height measurement

Results showed an increase in the crest level above the original level.
Diagrammatic representation of a tooth socket following extraction of teeth (left) and at the end of the healing period of the tooth socket after being filled with GranuMas™ (right)

The mean alveolar bone resorption before extraction and 6 months Post extraction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Height before (mm)</th>
<th>Height after</th>
<th>mean diff. (95% CI)</th>
<th>T-statistic (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRANUMAS</td>
<td>33.32 (4.80)</td>
<td>34.64 (4.32)</td>
<td>1.32 (0.78, 1.89)</td>
<td>4.95 (31)</td>
</tr>
<tr>
<td>Control</td>
<td>32.77 (3.8)</td>
<td>29.79 (3.83)</td>
<td>-2.97 (-3.47, -2.48)</td>
<td>-12.2 (31)</td>
</tr>
</tbody>
</table>
There was a significant difference in the mean alveolar bone height among the two groups. The data was analyzed using an Anova Test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Bone Ht. changes (mm)</th>
<th>F-statistics</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRANUMAS</td>
<td>32</td>
<td>0.38 (0.88)</td>
<td>15.09</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>-2.98 (1.37)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a significant difference in the mean alveolar bone height between the study groups (post-hoc comparison).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean difference (mm) (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRANUMAS vs</td>
<td>-4.84 (-7.50, -2.22)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Among the advantages of GranuMaSTM in dental applications are:

1. Biocompatible
2. User friendly
3. Builds up back the jaw bone to better able receive crown implants
**GranuMaS™ IN ORTHOPAEDIC APPLICATION**

*GranuMas™* is the first locally produced Class D medical device going into phase 2 clinical trials after going through an extensive series of in vitro and in vivo evaluation. The study involves young healthy adults who have had recent trauma resulting in a single closed fracture of the distal radius, proximal tibia or distal femur that requires bone grafting with no other complications. It follows GCP protocols with patient inclusion and exclusion criteria, Patient Information Packs regarding the proposed study in Bahasa Malaysia and English, Patient Informed Consent Forms in Bahasa Malaysia and English and a Patient Data Form for data collection. A series of X-rays for each patient for up to 1 year postoperatively that corresponds to the various phases of fracture healing to evaluate the effect of the product for the specific fractures used in this study as defined below:

- Pre-operative X-ray of injured site
- Day 1 post surgery
- 2 weeks post surgery (period of callus formation)
- 6 weeks post surgery (period of consolidation and radiological bone union)
- 12 weeks post surgery (period of clinical bone union)
- 6 months post surgery (period of early remodelling)
- 1 year post surgery (period of further remodelling)

**Inclusion Criteria**
Young healthy adults who have had recent trauma resulting in a closed fracture that requires bone grafting with no other complications

**Exclusion Criteria**
Below 18 years old or more than 40 years old
Known to have diseases that affect the quality of bone produced
Polytrauma patients
Unable to sign the informed consent form by themselves
Medical conditions that have a poor prognosis, low survival rates or poor healing ability
On-going osteomyelitis
Long-term steroid use
Known malignant neoplastic disease

A total of 50 patients has been implanted with *GranuMaS™* in a period of 9 months from August 2005 till May 2006.
Case 1 – Distal Radius Fracture

Pre-op X-ray indicates distal radius fracture

Immediate post-op AP view x-ray  Immediate post-op lateral view x-ray
Case 2 – Distal Radius

Immediate Post-Operative X-ray

Follow Up at 3 Weeks
Case 3 – Proximal Tibiae

Pre-Operative X-ray

Immediate Post-Operative X-ray
Follow Up at 12 Weeks

Follow Up at 6 Months
Case 4 – Distal Radius

Post-Operative X-ray

Good wrist function at 6 months follow-up
Case 5 – Open Distal End Radius Fracture with Bone Loss

An 18 year old male had an alleged motor vehicle accident and sustained an open fracture of the distal end of the right radius with bone loss. Patient was put on external fixator for 3 weeks post debridement before a second surgery was performed to remove the external fixator. Internal fixation of the fracture was performed in the same sitting and 3 g of GranuMas™ was also applied.

Pre-Operative X-ray

Immediate Post Debridement X-ray showing bone loss
**Implantation Surgery**

Exposure of fracture area

Insertion of GranuMaS™ in filling bone defect

Metal plate was used to stabilize the fracture

Further insertion of GranuMaS™ once the metal plate has been fixed

Post operative X-ray after internal fixation and implantation with GranuMaS™
Follow up at 6 weeks

Follow up at 12 weeks
Case 6 - Distal Femur fracture

Results for the Orthopaedic Application of GranuMaS™

Patients in this series have been followed up for a period of between 6 months to 1 year while further follow up visits are still on-going. All patients recovered well with no major complications. There was 1 case of superficial infection in the patient who was on the external fixator for 3 weeks prior to surgery due to an open fracture with bone loss (Case 5). However the infection cleared up after a course of antibiotics with no further complications. No collapse of fracture was seen. There was abundant callus formation with excellent union of the fractures seen.

As a conclusion, GranuMaS™ is suitable for use in patients with traumatic fractures and bone loss.
PUBLICATIONS & PRESENTATIONS

The results of the research and development of GranuMaSTM has been published and presented in the following journal and meetings:

Publications


Papers Presented


3. KA Khalid. Hafiz A., Bone Graft Substitutes – Research & Development in IIUM. Weekly CPC, Kulliyyah of Medicine, IIUM, Kuantan. 30 December 2005

4. KA Khalid. Phase II Clinical Trial Seminar on Synthetic Bone Tissue Transplantation: Research and Development – The Malaysian Experience, Le Meredien Hotel, KL Sentral, Kuala Lumpur. 21 November 2005


8. KA Khalid. *Animal Implantation Study and Planned Phase 2 Clinical Trials.* Orthopaedic Research Seminar 2005, Kulliyyah of Medicine, IIUM, Kuantan, Pahang. 6th May 2005


10. KA Khalid. *Orthopaedic Biomaterials Research in Malaysia.* Presented at the Malaysia-Thailand Joint Workshop on Advanced Materials, Advanced Materials Research Centre (AMREC), SIRIM Berhad, Kulim, Kedah. 6 – 7 September 2004


13. KA Khalid, *Orthopaedic Research in IIUM.* Presented at the East Coast Orthopaedic Meeting, Perdana Beach Resort, Kota Bharu, Kelantan. 13 January 2004


18. KA Khalid. *Biomaterials in Orthopaedics*. Presented at the Seminar on Basic Biomaterials Science, School of Dental Sciences, USM, Kubang Kerian, 26 – 27 March 2003


20. KA Khalid. *Biomaterials Research in IIUM – The Direction We Are Going*. Presented at the 4th National Colloquium on Biomaterials, Kulliyyah of Medicine, IIUM, 24th – 25th October 2002


22. KA Khalid and F Fazan. *Hydroxyapatite (HA) as a Bone Graft Substitute – Preliminary Results from Malaysia*. Proceedings of the 3rd World Congress on Tissue Banking, Boston, USA. 24 - 28 August 2002

23. KA Khalid. *Pilot Study on Hydroxyapatite (HA) as a Bone Graft Substitute*. Presented during the Presentation of Research Findings by The Winners of Dr. Ranjeet Bhagwan Singh Medical Research Grants, Institute of Medical Research, 25 June 2002


26. KA Khalid. *Biomaterials in Orthopaedics.* Presented at the Orthopaedics Basic Science Course, HUKM, September 2000


Acknowledgement

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1. Dr. Idris Besar  
   Malaysian Institute of Nuclear Technology Research (MINT)

2. Dr. Fazilah Fazan  
   Advance Materials Research Centre (AMREC), SIRIM Bhd

3. Assoc. Prof. Dr. Md. Anuar Osman  
   National University of Malaysia (UKM)

4. Prof. Dr. Abdul Rani Samsudin  
   Universiti Sains Malaysia (USM)

5. Asst. Prof. Dr. Kamarul Ariffin Khalid  
   International Islamic University Malaysia (IIUM)
GranuMaS™

**Product Name**
GranuMaS™
Hydroxyapatite granular bone graft.

**Brief Description**
GranuMaS™ is an osteoconductive granular synthetic bone graft material based on calcium phosphate hydroxyapatite. It is an excellent alternative material for the repair of bone defects due to its chemical composition being similar to the mineral phase of human bone. It is also chemically similar to the hydroxyapatite that is currently being used clinically as a bioactive coating on many surgical and dental implants. GranuMaS™ is invented and produced by the Advance Materials Research Centre (AMREC) of SIRIM Berhad using a patented process [Malaysian Patent Pending P1 2004 0748]. It is derived from pure commercial chemicals and Malaysian limestone and has fulfilled all of the criteria required under the ASTM F1185-88 (1993) Standard for Composition of Hydroxyapatite (HA) for Surgical Implants. This product has gone through extensive biocompatibility and safety evaluation and has also demonstrated excellent biofunctionality in clinical trials, which were all conducted with the collaboration of respectable institutions of higher learning in Malaysia.

**Material**
GranuMaS™ is a synthetic, osteoconductive and biocompatible calcium phosphate bioorganic that consists of >99% pure hydroxyapatite \([\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2]\) phase. It conforms fully to the US standard ASTM F1185-88 (1993) (Composition of Hydroxyapatite (HA) for Surgical Implants). GranuMaS implants are offered in 4 different granule size range (200 – 500 µm, 500 – 1000 µm, 1000 – 2500 µm and 2500 – 5000 µm) and provided gamma sterilised in a triple sterile pack.

**Application Fields and Indications**
GranuMaS™ can be used in surgeries requiring bone grafting for non-loading bearing applications such as in the Dental, ENT, Orthopaedics and Maxillofacial specialities. Among the use that GranuMaS™ is recommended for are:
- as a filler of bone defects
- reconstruction of damage or resected regions of bone
- fusion of the joints or vertebra
- as a bridge or spacer of regions with bone loss
- augmentation of osteoporotic bone defects
- the augmentation, correction and rectification of malpositioned bone

**Contraindications**
GranuMaS™ should not be used in patients with:
- acute and chronic infections in the surgical area, including and not restricted to soft tissue infections, posttraumatic infections, chronic osteomyelitis, inflammatory and bone diseases
- untreated primary malignancy of bone and other musculoskeletal tissues, malignant myeloma, Burkitt’s lymphoma and other lymphomas

**Relative Contraindications and Restrictions of Use**
Depending on the risk-benefit assessment GranuMaS™ could be used in patients with:
- serious metabolic disorders, such as severe refractory Diabetes Mellitus patients
- impaired calcium metabolism
- steroid or other pharmaceutical therapy that adversely affects calcium metabolism
- immunosuppressive drug therapy
- severe or endocrinologically-induced bone disorders (e.g. hyperparathyroidism)
- poor bone healing
- an open epiphyseal growth plate

In such instances, it is important and ethically correct to inform the patients that the above conditions may affect the progression and final outcome of the planned intervention. It is also recommended that the patient be inform regarding the possibility of some activities that may reduce the effects of these aggravating conditions.

**Caution**
GranuMaS™ should only be used by, or under the supervision of, trained medical personnel with sufficient training experience in the required surgical techniques and the field of biomaterials.

**Side Effects**
No known side effects of the material or hydroxyapatite are known to date.

**Interactions**
No interactions between the material or hydroxyapatite with other pharmaceuticals or medical products are known.

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**Dosage**
GranuMaS™ comes pre-packaged in quantities of either 0.5g, 1g, 2g, 5g or 10g depending on the available granule size range. The amount use will depend on the size of the defect to be treated. The amount used should be press-fitted to completely fill the bone defect. It is not advised to overfill the defect as this can cause tension of the wound closure.

**Method of Administration**
GranuMaS™ is available in the following granule size range and amounts:

<table>
<thead>
<tr>
<th>Size</th>
<th>0.5g</th>
<th>1g</th>
<th>2g</th>
<th>5g</th>
<th>10g</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 – 500 µm</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>500 – 1000 µm</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>1000 – 2500 µm</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2500 – 5000 µm</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

GranuMaS™ should always be applied in direct contact with viable bone tissue. The decision to use GranuMaS™ during any surgical procedure, as outlined in the Application Fields and Indications section, will depend on the experience of the surgeon. The choice of granule size and amount to be used will depend on the application area and the nature and extent of the defect to be addressed. The bone defect should be completely filled with the material and primary wound closure should always be performed. Care must be taken to avoid too much compaction in the bone defect.

GranuMaS™ is ideal for use especially for defects in the periacrural region were early cross-sectional strength is required as the material has been shown to be highly osteoconductive and able to help in the formation of mature bone across the defect.

Direct contact with bleeding viable bone tissue is critical for GranuMaS™ to fully function as an osteoconductive material. Therefore freshening of the bone prior to application is required in some cases. Similarly, cells and factors involved in bone regeneration and growth must be present in order to promote bone healing upon and after application. Non-viable bone splinters and necrotic tissue must be carefully removed prior to application. It is recommended to mix GranuMaS™ with autologous bone marrow during application. For larger sized defects, requiring the use of more than 10g of material, mixing with autologous bone material is also highly recommended.

GranuMaS™ is not meant to perform load bearing functions. Therefore mechanical stability of the site should be achieved before applying GranuMaS™ into the defect. The additional osteosynthetic measures required depends on the type and location of the bone defect to be treated. GranuMaS™ can also be used as an adjunctive material for intervertebral fusion in spinal surgery.

**Particular Points To Note**
- GranuMaS™ is radiopaque
- GranuMaS™ does not contain any unregistered, illegal or “non-halal” substances
- GranuMaS™ has no load bearing function
- Do not re-sterilise GranuMaS™
- Unused granules should be discarded

**Handling**
GranuMaS™ product is triple sterile-packed. The contents of the tube and inner packaging are sterile. The inner packaging must not be open until required for use. Remove the sterile tube from the opened inner packaging and apply the material for use as intended.

These granules must not be used if the packaging is visibly damaged.

GranuMaS™ should not be re-sterilised

GranuMaS™ must not be used once the expiry date specified on the container and outer wrapper / box has expired

NB. The product can only be returned if the seal on the protective packaging is still intact

**Documentation**
It is recommended to attach the enclosed stickers to the patient’s bed-chart and operation notes and also to the operation theatre’s documentation to enable traceability of the product at any given time.