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A Novel Synthetic Dental and Bone Graft Substitutes: A Proof of Concept Prototype

Dr. V. Madhusudhana Rao
BDS, Dental Surgeon, ESI Diagnostic Centre, Jeedimetla, Hyderabad, India
Dr. K. Srinivas Rao
MS - Orthopaedics, Civil Surgeon – Orthopaedics, ESI Hospital, Sanathnagar, Hyderabad, India

Abstract:
Dental and Bone graft substitutes are used in Dental surgery, orthopaedic surgery and Neuro - surgery. . They are used in filling defects or voids resulting from bone loss due to trauma or deformity. Since decades autograft, allograft and mineral bone graft like Calcium phosphate, Calcium sulphate, Tri Calcium phosphate are being used. A novel bone biomaterial which is a nanocomposite of ECM and HA has been developed. ECM provides the body's own natural bone growth factors to supplement the action of the bone graft.
We prepared an ECM emulsion with a suitable organic solvent and blend it with simulated body fluid (SBF) for deposition of nano crystals is the first step. After initial evidence we optimized the SBF and ECM composition for faster nucleation and HA nano crystal growth. We are hypothesizing that the non-collagenous gap junction between tropocollagen in the collagen fibrils will be sufficient by optimizing collagen concentration to induce nucleation of HA and achieve high strength in short time. To study the physical characterization of ECM-HA formulations (freeze dried sample) SEM and XRD were used.
The results showed that the materials mated successfully with evidence of HA
Deposition of ECM as demonstrated by Scanning Electron Microscope (SEM). The X ray diffraction shows proper crystal deposition.
This novel biomaterial which is an ECM-HA nanocomposite can address the downsides of autograft, allograft and existing synthetic bone substitutes. Such a novel bilateral composite will be a common substitute for multiple clinical needs as it can create most of the device formats like cortical, cancellous, granules, paste etc.
It can be developed as a therapeutic range of class I predicate devices for various bone defects managements

Key words: *Biomaterials, ECM, HA, Novel, Nanocomposite*

1. Introduction

Dental surgeons, Orthopaedic surgeons, and Neurosurgeons face many challenges while managing surgeries, especially when bone grafting is required. Bone graft is the second most common transplanted tissue, with blood being number one. [1] More than 2 million bone grafting procedures are performed annually for the repair of bone defects in Orthopaedics, neurosurgery, and dentistry. [2-4] approximately 10% of all skeletal surgical interventions require bone grafting. 4Large defects resulting from trauma, infection and tumour resection often do not heal spontaneously, and require bone grafting.
Costs relating to fracture, spinal fusions, and replacement of hip and knee joint was estimated to be over $20 billion in 2003, and predicted to increase to over $74 billion by the year 2015. Many of those surgeries require bone grafting.
Traumatic bone fractures accounted for 8.5 million operations every year, almost 1 million of which requires bone grafting/ substitutes. Spinal arthrodesis is an example of a surgery typically requiring substantial bone repair/ replacement. In US alone over 300,000 spinal fusions are done. Around 3000 paediatric hospitalizations for bone cancer require bone The autograft[5] and allograft procedures are mostly successful. It is attributed to the physical and biological similarity in donor (site or patient) and host tissue. Autologous bone needs to be harvested from a donor site and is associated with a risk of morbidity and is available only in limited quantities. [7,8,9]Moreover, the use of autografts is not recommended in elderly or pediatric patients or in patients with a malignancy or infectious disease. Osteocytes in autologous bone graft may not survive transplantation, the clinical benefit is not guaranteed per se [6]

The allogeneic bone another viable option. There are a number of orthopaedic allograft products which have been FDA-approved and utilized for years. However, orthopaedic allografts carry risks of donor to recipient infection (rate of incidence as high as 13%) and host immune responses. [8]

The bone xenografts are now widely considered as unsuitable for transplantation [8] due to risk of infection, toxicity associated with sterilization, immunogenicity, and finally host rejection [15, 16]

The safe and abundant bone substitute appears to be a synthetic material like calcium phosphate, Calcium sulphate etc. Synthetic materials were the focus of R&D for commercial development. Many (artificial) bone substitute materials are currently available for use in orthopaedic surgery. Various biomaterials including Calcium sulphate [9,] Calcium phosphates as tricalcium phosphate and Hydroxyapatite [10,] metals, polymers, and composites have been investigated for their potential as bone substitute materials [11, 12, 13]

The ideal dental or bone substitute should have the following characteristics:

- Provide temporary mechanical support to the affected area
- Act as a substrate for osteoid deposition
- Contain a porous architecture to allow for vascularization and bone in-growth
- Encourage bone cell migration into the scaffold
- Promoteosteogenic differentiation (osteoinduction)
- Enhance cellular activity towards scaffold-host tissue integration (osseointegration)
- Degrade in a controlled manner to facilitate load transfer to developing bone
- Produce non-toxic degradation products
- Should not incite an active chronic inflammatory response
- Capable of sterilization without loss of bioactivity.

The ceramic materials like hydroxyapatite have certain downsides when it comes to their plasticity, [21] in the context of their interaction with the matrix (Collagen) in tissues. [23] They are difficult to be processed as porous bone structures and lack hierarchical organization of natural bone at nanometre scale another limitation, however, of bulk CP materials is their brittle nature and poor mechanical properties. [14] As a result, these materials have been used clinically only in non-load-bearing indications, primarily as granules and blocks. The inability to sculpt the bulk materials to conform to irregular defects and the possibility of the granules migrating from the implant site has led me to this formulation of self-setting calcium phosphate (H A) with Extracellular matrix (ECM.) These materials set by a precipitation and can be moulded into desired shapes or injected into defects in minimally invasive procedures.

Extracellular matrix (ECM) based products are now well accepted in a number of clinical situations as predicate devices. Handling and customs processing of ECM in the required physical formats like powder, blocks, monoliths, paste etc. are already satisfactorily achieved ECM inherently does have reasonably robust physical and biological properties required to handle cells. [17] Engineered extracellular matrixes is one of the thrust areas across the world to deliver regenerative medicine solutions. ECM can be customized to provide a tissue specific microenvironment. Since early 80's success of extracellular matrix based devices have proven itself by completing research to marketplace cycle many a times. Extracellular matrix based devices have entered into the next generation of evolution. I am hoping to custom integrate both ECM and HA with various engineering and biological attributes in a single biomaterial technology platform for realistic applications as cancellous bone, cortical bone, cortical/cancellous bone.

The intimate nano assembly of hydroxyapatite with collagen giving rise to Havervsian canals is a dream considering biomaterial technology paradigm today.

2. Objective

A proof of concept prototype for synthetic bone substitute consisting of a nano composite of extracellular matrix and hydroxyapatite to create an ideal solution for synthetic bone substitute provided the current standards of benchmark products could be met through appropriate formulations.

Such an ECM-HA composite will be a common substitute for multiple clinical needs.

3. Materials and Methods

We have developed a process for assembling complex extracellular matrix architecture (spatial arrangement of ECM polymers) and blending it with HA. The process is novel and scalable. We are hoping to custom integrate both engineering and biological attributes in a single biomaterial technology platform for realistic applications [23]

The mere presence of ECM will boost the osteoinductive and osteogenic properties of the graft material ECM provides a "dose" of the body's own natural bone growth factors to supplement the action of the bone graft.[26]

Existing bone substitute material	Brand/Companies	Features of Novel ECM-HA Composite
Cancellous / Cortical Bone Allograft	Puros/ Zimmer	ECM-HA can be delivered as putty, powder, granules, monoliths, injectable paste etc.
Bioglass	Cortoss/ Orthovita	
Calcium sulphate	MIIG X3/ Wright Medical	
□-tricalcium phosphate	BoneSave/ Stryker	
Porous (Coralline) HA	ProOsteon/ InterporeInt	
Injectable HA putty	Norian SRS/ Synthes	
Bone Cement (PMMA)	CMW1/Depuy	

Table 1

Challenging aspect of this formulation is to prepare such emulsions of collagen and chemically deposit nano phase hydroxyapatite in gap zones of tropo-collagen to examine the mimicry of natural hydroxyapatite formation in the collagenous environment. and to achieve hierarchical hydroxyapatite nucleation in the tropo-collagen fiber intervals.
The Major technical challenge for such nano composite formulation may lie in achieving reasonable mechanical strength of natural bone *in situ*immediately after surgery to facilitate patient movement instead of lengthy resting phase.
Preparation of ECM emulsion with a suitable organic solvent and blend it with simulated body fluid (SBF) for deposition of nano crystals is the first step. After initial evidence we optimized the SBF and ECM composition for faster nucleation and HA nano crystal growth. We are hypothesizing that the non-collagenous gap junction between tropocollagen in the collagen fibrils will be sufficient by optimizing collagen concentration to induce nucleation of HA and achieve high strength in short time. The sample is prepared by freeze drying after removing the solvents
To study the physical characterization of ECM-HA formulations (freeze dried sample) Scanning Electron Microscopy (SEM) and X Ray Diffraction (XRD) were used. Electron microscopy study to enumerate HA deposition and X-Ray diffraction for proper crystal composition.

4. Results
The results showed that the materials mated successfully with evidence of HA deposition on ECM as demonstrated by SEM. The X ray diffraction shows proper crystal deposition. ECM mimics the organic component of bone and it predominantly contributes to the tensile strength of this nanocomposite. ECM remained cytocompatible after processing. ECM scaffolds which were used are free of pyrogens and toxins. The biological and physical properties of the ECM are not lost in the processing of nanocomposite.
The degradation process can be controlled by varying the proportions of ECM and HA.

5. Discussion
Bone graft substitutes find application in augmenting/ enhancing the healing of fractures and fusions and in filling defects or voids resulting from bone loss due to trauma or deformity. [22]They are used in most spinal fusion and revision joint replacement procedures [20]and in many oncology, fracture repair and melanin/ non-union [22]applications, as well. Currently, orthopaedic surgeons have several technology options available for their bone grafting needs – autograft, allograft, xenograft and synthetics.
To address the downsides of autograft and allograft, companies worldwide has developed synthetic materials, the most widely used being calcium phosphates, calcium sulphates and hydroxylapatites (HAs) [28]These materials provide osteoconductive scaffolding onto which new bone may grow and can also serve as delivery vehicles for osteoinductive and osteogenic substances. [25]
Hydroxyapatite and ceramic are the material of choice due to high strength in clinical practice, for example, calcium phosphate cements (BoneSource®, Calcibon®, ChronOS®®, Eurobone®, HydroSet™, NorianSRS® and Ostim®), Calcium sulphate cement (MIIG® X3), Bioactive glass, cement (Cortoss®). [24]These materials have certain limitations when it comes to their plasticity in the context of their interaction with the matrix (Collagen) in tissues. They are difficult to be processed as porous bone structures and lack hierarchical organization of natural bone at the nanometre scale. Another downside of Calcium Phosphate materials is their brittle nature and poor mechanical properties. [23]
In order to overcome the drawbacks of mineral bone graft23 substitutes a nanocomposite of ECM and HA has been developed. The ECM will boost the osteoinductive and osteogenic properties of the graft material ECM provides a “dose” of the body’s own natural bone growth factors to supplement the action of the bone graft. The 27Engineered extracellular matrix is one of the thrust areas across the world to deliver regenerative medicine solutions. Since early last three decades success of extracellular matrix based devices have proven itself by completing research to marketplace cycle many a times. Extracellular matrix based devices have entered into the next generation of evolution
Such an ECM-HA composite will be a common substitute for multiple clinical needs as it can create most of the device formats like cortical, cancellous, granules, paste *etc.*
It has potential to develop a therapeutic range of class I predicate devices for various bone defects management

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7. References

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