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Research Article

A Proof of Concept Prototype for Novel Synthetic Bone Substitute Consisting of a Nano Composite of Extracellular Matrix and Hydroxyapatite

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Abstract: Bone graft substitutes are increasingly being used by Orthopaedic surgeons, Dental surgeons and Neurosurgeons to fill up the bone loss and defects resulting from trauma and deformity. Autografts, allografts and mineral bone substitutes are being used for bone grafting. To address the downsides of autograft, allograft and mineral bone graft we developed a novel bone substitute using the proprietary ECM technology platform and nano Hydroxyapatite. The ECM component of the nano composite provides natural growth factors. It will boost the conductive and osteogenic properties of the bone graft. Deposition of nano crystals of Hydroxyapatite atthe inter tropocollagen junction is the first step. We prepared anECM solution with suitable organic solvent and it is blended with simulated body fluid (SBF) for deposition of crystals ofHydroxyapatite Optimization of ECM emulsion and SBF was done after achieving hierarchal nucleation and growth of HA crystals. Scanning Electron Microscopy (SEM) and X-ray diffraction (XRD) were used to characterize the nano composite of ECM and HA. The Scanning Electron Microscopy (SEM) demonstrated the HA deposition and pore size was found to be adequate for migration of osteoblasts. Calcium crystal deposition was evident in X- ray diffraction (XRD) data. These novel bioceramic devices are developed by nanostructure processing of inorganic and organic biomaterial with ultra fine structure. These devices with excellent mechanical and biological properties are for orthopaedic and dental implant applications. This promising technology can address the downsides of existing bone graft substitutes viz., autograft, allograft and synthetic bone substitutes. Moreover multiple device formats like cortical, cancellous, granules, paste etc can be fabricated from this novel technology. All these devices are considered as predicate as per USFDA and route to the market is faster.
Keywords: ECM, HA, Novel, Nanocomposite

INTROUCTION

Orthopaedic surgeons, Dental surgeons and Neurosurgeons are faced with challenges while managing bone defects arising out of trauma and deformity. Treatment of bone defects is a major challenge for Orthopaedic surgeons. Bone graft is one of the most commonly transplanted tissues in humans after the blood [1]. Globally more than 2.2 million bone grafting procedures are done every year [2-4]. Large bone defects do not heal spontaneously and require bone grafting procedures.

Dr K.S. Rao's staging of bone engraftment:
Stage 1 - Provide temporary mechanical support to the affected area
Stage 2 - Act as a substrate for osteoid deposition
Stage 3 – Osteo-conduction bone cell migration into the graft
Stage 4 - Host tissue integration (osseo-integration)

Medical expenses relating to fracture, spinal fusions, and replacement of hip and knee joint was estimated to be over $20billion in 2003, and predicted to increase to over $74 billion by the year 2015. Many of those surgeries require bone grafting orbone substitutes to fill up bone defects.

Traumatic bone fractures accounted for 8.5 million operations every year, almost 1 million of which requires bone grafting orsubstitutes. Spinal arthrodesis is an example of a surgery typically requiring substantial bone repair or replacement. In US alone over 300,000 spinal fusions conducted every year costing over $20 billion (USD). Around 3000 pediatric hospitalizations for bone cancer require bone grafting or substitutes costing over $70 million Success in both autograft [5] and allograft procedures is attributed to the physical and biological similarity in donor or patient) and host tissue. Autologous bone was the bone substitute need to be harvested from a donor site.

Autograft bone was typically harvested from iliac crest. Besides, limited availability it is associated with the risk of donor site morbidity and infection [6]. Autografts cannot be harvested from geriatric, pediatric patients and those suffering from malignancy and infection. Osteocytes in autologous bone graft may not be viable after transplantation [7]. Another reasonable option for bone substitute is allogenic bone. Many orthopedic allograft procedures have been FDA-approved and utilized for years. However, orthopedic allografts carry risks of donor to recipient infection (rate of incidence as high as 13%) and host immune responses [8].

After more than two decades of global investigation and clinical trials, bone xenografts are now widely considered as unsuitable for transplantation due to risk of infection, toxicity associated with sterilization, immunogenicity, and finally host rejection [6, 8]. Only alternative for a safe and abundant bone substitute appear to be a synthetic material. Indeed synthetic materials have been the focus of R&D for commercial development. Many synthetic bone graft substitutes are available for the management of bone defects [9]. Various biomaterials including Calcium sulfate, Calcium phosphates, Tri-calcium phosphate, Hydroxyapatite, metals, polymers and composites have been studied for their use as bone graft substitutes [10-13].

Dr K.S. Rao's Ten Commandments for an ideal bone substitute;

- 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
- Promote osteogenic differentiation (osteo induction)
- 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 including hydroxyapatite have certainlimitations when it comes to their plasticity [21] in context of their interaction with matrix (collagen) tissues. These materials are difficult to be processed as porous bone structures and lackhierarchical organization of natural bone at nanometer scalelimitation, 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 (HA) with Extracellular matrix (ECM) [15, 16]. These materials set by a precipitation and can be molded to 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 [16]. Handling and customize processing of ECM in the required physical formats like powder, blocks, monoliths, paste etc. are satisfactorily achieved. ECM inherently does have reasonably robust physical and biological properties required to handle cells [17].

Engineered extracellular matrix is one of the thrust areas across the world to deliver regenerative medicine solutions. ECM can be customized to provide 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 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 cortical bone, cortical or cancellous bone. The intimate nano assembly of hydroxyapatite with collagen giving rise to Havervsian canals is a dream considering biomaterial technology paradigm today.

Objectives

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 as ECM-HA nano composite will be a common platform for fabricating multiple device formats for various clinical needs.

MATERIALS AND METHODS

We have developed a process for assembling complex extracellular matrix architecture (spatial arrangement of ECM polymers) and blending it with HA. 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.

ECM based products have been classified as GRAS (Generally regarded as Safe) by USFDA. They have been approved as Predicate devices by USFDA. The ECM component of the nano composite provides natural growth factors. It will boost theconductive and osteogenic properties of the bone graft [18].

Table 1: List of existing bone graft substitutes

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 Sulfate	MIIG X3/ Wright Medical	
β-tricalcium phosphate	BoneSave/ Stryker	
Porous(Coralline) HA	ProOsteon/ Interpore Int	
Injectable HA putty	Norian SRS/ Synthes	
Bone Cement (PMMA)	CMW1/Depuy	

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 fibre intervals.

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. With high internal phase emulsion, it is possible for hierarchical hydroxyapatite nucleation in the tropo-collagen fiber intervals [19, 20].

Reconstituted ECM emulsion in a suitable organic solvent is blended with simulated body fluid (SBF). After initial evidence of crystal deposition, the formulation was optimized for faster Nucleation of HA crystal growth. The sample is prepared by freeze drying after removing the solvents. Scanning Electron Microscopy (SEM) and X Ray Diffraction (XRD) were used tocharacterize the sample. Electron microscopy was used to measure the pore size and enumerate HA deposition. X-Ray diffractionwas for proper calcium crystal composition.

RESULTS

Scanning Electron Microscope data, Fig. 1 showed HA deposition on ECM and pore size was found to be right for migration of osteoblasts. XRD, Fig. 2 clearly demonstrates the presence of calcium crystals.

ECM mimics the organic component of bone and it predominantly contributes to the tensile strength of this nano composite. ECM remained as 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

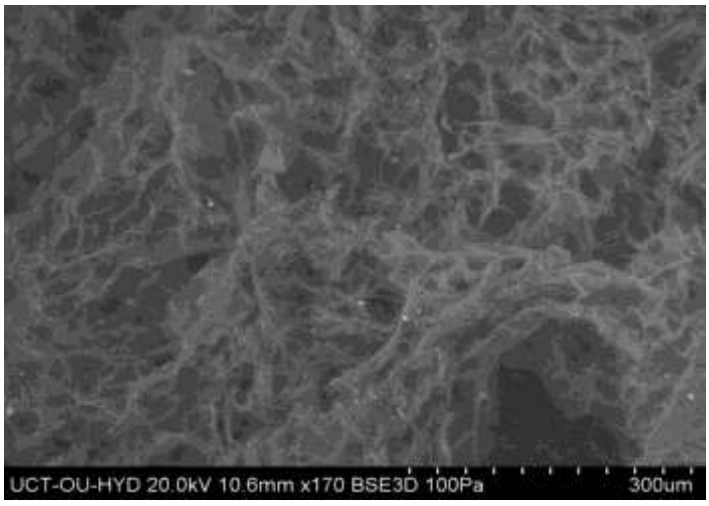

Fig 1: SEM Photographs showing HA Deposition on Extra Cellular matrix

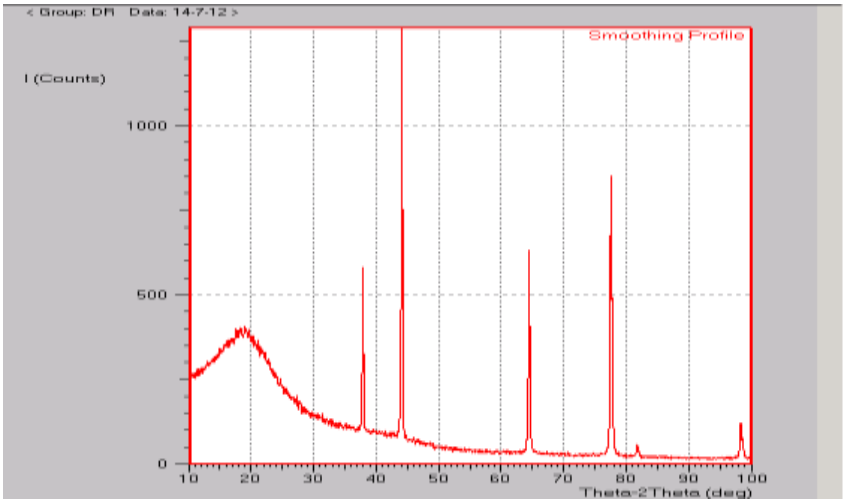

Fig. 2: X-Ray Diffraction showing calcium deposition

DISCUSSION

Bone graft and substitutes help Orthopedic surgeons, Dental surgeons and Neurosurgeons in filling up the bone loss and defects due to trauma and deformity. Similarly it is being increasingly used in spinal fusion procedures and joint replacement surgeries [20].

There are several options available for surgeons like allograft,autograft and synthetic substitutes. Synthetic bone substitutes like calcium phosphate, calcium sulfate and hydroxyapatite are being increasingly used to avoid the downsides of autograft and allograft [21-23]. These synthetic bone substitutes provide the scaffolds for osteo connection into which new bone may grow [24].

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 sulfate cement (MIIG® X3), Bioactive glass cement (Cortoss®) [5]. These materials have certain limitations, when it comes to their plasticity in context of their interaction with matrix (Collagen) in tissues. They are difficult to be processed as porous bone structures and lack hierarchical organization of natural bone at nanometer scale. Another downside of Calcium Phosphate materials is their brittle nature and poor mechanical properties [25]. In order to overcome the drawbacks of mineral bone substitutes a novel nano composite made of ECM and HA has been formulated. ECM contributes to osteo conductive and osteogenic properties and provides bodies own natural bone growth factors [26].

Engineered 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 [26]. Extracellular matrix based devices have entered into next generation of evolution. Most of the device formats likecortical, cancellous, granules; paste etc can be fabricated from the reconstituted solution of ECM-HA nano composite. They have a huge potential to develop a whole range of class I and class II predicate devices for bone graft.

CONCLUSSION

A nano composite of ECM and HA has been developed to address the drawbacks of autologous, allogenic, xeno and mineral bone graft substitutes. ECM will boost the osteo-inductive and osteogenic properties of the graft material. Extracellular matrix isone of the thrust areas across the world to deliver regenerative medicine based therapies.

Our proposed ECM-HA composite can be a common bone 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 as per USFDA for various bone defects management.

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