GRAFTING OF METHACRYLATE POLYMERS ONTO PROCESSED BONE AND FIBRIN AS BIO-ACTIVE MATERIALS

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By

JESURIETTA KALAVATHANIE

Supervisor

Dr. GANGA RADHAKRISHNAN

POLYMER DIVISION
CENTRAL LEATHER RESEARCH INSTITUTE
ADYAR, CHENNAI 600 020, INDIA.

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INTRODUCTION

Macromolecular science is experiencing an explosive growth due to the important role macromolecular materials play in modern technology. Recently, there has been a growing interest in combining the useful properties of both natural and synthetic materials, to improve the quality of life in general and human life in particular[1]. Grafting is a convenient technique to modify natural polymers to get desirable properties without altering the core structure of the substrate.[2] These grafted bio-materials offer an enormous range of practical applications especially in the medical field.

Bone and bone-cements

Bone is made up of a porous frame work that is constantly rebuilding itself and contains *Hydroxyapatite* (HAp), $[Ca_{10}(PO_4)_6(OH)_2]$ as the major component, to the extent of 70% [3]. The enormous increase in skeletal reconstruction of the bone defects and joint replacements has led to the generation of bone-substitutes with better osteo-conductivity and bio-compatibility by modifying natural materials by graft-polymerizing them with synthetic monomers[4]. Bone-implants involving HAp are expected to cause few disturbances in the biological environment after implantation because of its close resemblance to the mineral matrix of bone[5]. Natural HAp from the bone of fish and mammals have been grafted with methacrylates and used as prosthetic materials.[6,7]

The introduction of self-curing acrylic *cement*, poly (methyl methacrylate), PMMA by Sir John Charnley[8] in 1970, for partial joint replacement, has revolutionized bone fracture repair. Since then, PMMA has emerged as a premier synthetic material in

contemporary orthopedics. But it has a lot of shortcomings - thermal necrosis, cracking of implant with time, loosening at the bone - cement interface etc[9]. Attempts are being made[10] to overcome these defects and develop bioactive PMMA bone-cements.

Fibrin and clot formation

In the blood plasma, fibrinogen is circulating along with the enzyme, prothrombin. When the blood vessels are torn during injury, the prothrombin gets converted to thrombin which catalyses the conversion of fibrinogen into fibrin by removing four low-molecular-weight peptides. Each molecule of fibrin automatically polymerizes within seconds and undergoes multiple cross-linking between adjacent fibers, forming a three-dimensional fibrin meshwork, commonly known as the *clot.*[11]

It is a common practice these days to use fibrin sealants during surgery since they function as tissue adhesives and aid in enhanced wound closure and healing by providing mechanical strength at the site of wound[12].

Scope of the present work

The focus of this work is to convert the under-utilized biomaterials into those of potential use by processing them from wastes and grafting biocompatible polymers on them, which apart from increasing the mechanical strength, introduce new functional groups to possibly couple with antibiotics. Also, these bioactive materials are inexpensive and so can be used by everyone. The present work aims to:

1. degelatanize industrial bone-wastes and compare the degelatinized bone (DGB) with synthetic HAp by Fourier-transform infrared spectroscopy (FTIR) and X-Ray diffraction (XRD) methods.

2. evolve a suitable method for grafting of Methylmethacrylate (MMA) onto DGB, study the effect of variables on graft-formation and optimize process parameters.
3. isolate the graft copolymer from the homopolymer and characterize the graft by analytical methods – FTIR, XRD, Scanning Electron Microscopy (SEM), particle- size analysis and porosity studies.
4. evaluate the use of the grafted material as bone filler by *in vivo* studies on rabbits.
5. isolate Fibrin from the slaughterhouse waste-blood.
6. graft-copolymerize fibrin with 2-hydroxyethyl methacrylate (HEMA) by free radical polymerization technique and characterize the graft.
7. study the wound healing ability of the fibrin graft on rodents.

CHAPTER II PREPARATION & CHARACTERIZATION OF DGB GRAFT

The bone-wastes from the bone-glue industry were washed, powdered and autoclaved to remove all the proteinous matter. This degelatinized material DGB was dried, finely powdered and sieved for uniform particle size. It was characterized for its functional groups by FTIR and crystal characteristics by XRD method and compared with those of synthetic HAp. DGB showed absorptions at the same frequencies in FTIR as synthetic HAp and also identical lattice parameters were observed from XRD analysis.

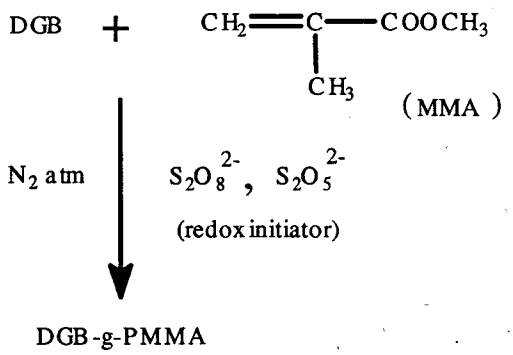
Preparation of DGB graft with PMMA

PMMA was grafted onto processed DGB as follows:

The Polymer grafting was achieved by radical polymerization of MMA by using redox initiators[13]. The DGB powder was soaked in water overnight. To this, sodium metabisulfite and potassium persulfate were added, followed by the addition of a known

amount of MMA. The experiments were performed at different pH values ranging from 3-9, at temperatures from 40°C to 100°C at different time intervals, as per Scheme1.

Scheme 1: Formation of DGB-g-PMMA



The crude gross copolymer was extracted with benzene to remove PMMA homo-polymer and the DGB-g-PMMA was dried and weighed. The percentage of grafting was calculated and the conditions for the formation of graft optimized. The graft copolymer was characterized by FTIR, TGA, XRD and SEM studies.

CHAPTER III EVALUATION OF DGB-g-PMMA AS A BIOACTIVE MATERIAL

Preparation of the implant material

The bio-polymeric material DGB-g-PMMA was kneaded into a dough using gelatin as a binder, compacted and extruded through a glass tube. The cylindrical grafts were air-dried, packed in polythene bags and sterilized by γ irradiation at 2-3 M rads.

Surgical Procedure

Eight one-year-old rabbits were anaesthetized and longitudinal incision was made on the tibia and the proximal end exposed. Using an implant-motor at a speed of 5000 rpm, a hole was made. Both the legs were used for this study. In the left leg of the animals, the defect was not packed with any material. In the right leg, the sterile gap was

packed with the DGB-g-PMMA graft and the wound closed by suturing the connective tissue and skin in separate layers.

The rabbits were well-maintained and fed with fresh feed and water *adlibitum* and the onset of bone-formation was assessed at the end of 3,6,9 and 12 weeks.

Radiology Results

Control (left leg): The 3^{rd} ,6^{th}, 9^{th} weeks' post-operative radiographic findings revealed the site-defect in all the animals since no radio-density changes were observed. However 12^{th} week radiograph revealed slight radio-density indicating only mild callous formation.

Treated (right leg): The 3^{rd} week radiograph showed retention of fragments although no radio-density changes were observed. By the 6^{th} post-operative week itself, there was increased radio-density at the fractured ends indicating mild callous formation. After the 12^{th} week, there was satisfactory callous formation between the two cortices.

Osteomedullography Results

Control (left leg): The dye injection on the 6^{th} and 9^{th} week, showed non-uniform flow of the dye due to leakage through the bony defect indicating absence of callous formation. However the 12^{th} week observation showed a fairly uniform flow of the dye indicating partial sealing of the bone defect.

Treated (right leg): A regular flow of the injected dye right from the 9^{th} week onwards, clearly proved enhanced bone growth when the bony defect is sealed with polymer graft. It was obvious that the defects filled with the graft showed excellent osseous growth as compared to the control. Since there was no rejection or inflammation at the site, the DGB-g-PMMA could be considered for further clinical trial.

CHAPTER IV PREPARATION & CHARACTERIZATION OF FIBRIN GRAFT

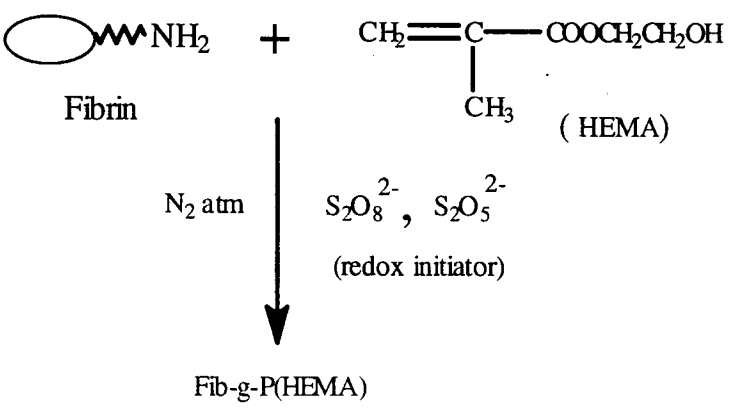
Processing of Fibrin

Bovine blood from slaughterhouse was immediately churned at a speed of 40 rpm when the fibrin separated out as threads, which were washed with running water and cleaned thoroughly and soaked in a solution of sodium acetate to remove any remaining blood stain. The pH was adjusted to 8.5 by adding a few drops of NaOH. Then H_2O_2 was added with stirring when the fibrin became white and frothy. The material was washed with water till the pH reduced to7 , ground moderately in a blender and stored in a freezer.

Preparation of Fibrin-polyHEMA graft polymer (Fib-g-P(HEMA))

Fibrin was graft-polymerized with poly (2-hydroxyethyl methacrylate) [P(HEMA)] as follows: The fibrin was suspended in water in a three-necked flask fitted with a motorized stirrer. The redox initiator solutions, sodium metabisulfite and potassium persulfate were added, followed by the addition of HEMA and heated in a water bath in a nitrogen atmosphere, as per Scheme 2. The crude graft was filtered, dried and weighed which was then extracted with acetone to remove the homopolymer, P(HEMA) and the weight of the graft noted. The grafting of the polymer on Fibrin was proved by FTIR and SEM studies.

Scheme 2 : Formation of Fib-g-P(HEMA)



CHAPTER V FIBRIN-g-P(HEMA) AS A BIOACTIVE MATERIAL

Invivo study of Fib-g-P(HEMA) for Wound healing

In order to evaluate the additional benefit of grafting a synthetic polymer on the biopolymer in the healing of wounds, animal study was conducted.

Open-wound Study:

Eighteen male Albino rats, were chosen for this study and divided into three groups of six animals each- a) control b) fibrin c) fibrin-g-P(HEMA).

Each animal was given general anesthesia under sterile conditions and shaved under aseptic conditions. A wound was created on its back by removing a square piece of skin (2x2cm.) and the wound treated as per the grouping. The animals were maintained in individual cages and fed with fresh feed and water *adlibitum.* The wounds were cleaned and the drugs applied every morning. On the 4^{th}, 8^{th}, 12^{th} and 16^{th} days, the animals were photographed, the wound-area traced out for measurement, the scabs formed were carefully removed and preserved for collagen estimation by biochemical analysis.

Incision-wound Study:

Another set of 18 male Albino rats were grouped as before. After anesthetization, the back of the animals was shaved and incision wounds 6 cm. long were created and sutured with 5 stitches with a sterile needle and thread. While the control group was daubed with disinfectant alone, the other two groups were treated daily with the corresponding materials. After 8 days, the skin samples from the incised areas were harvested and preserved for histopathological studies.

The animals of the incision-wound study showed healing by 8 days, while open-wound took 16 days to heal completely. All the animals recovered quickly and did not show adverse tissue reaction around the implants.

Results of the Open-wound Study:

The enhanced wound-healing trend of the *treated* wounds as compared to the *control* was evident from the examination of the photographs and biochemical analysis.

Results of the Incision-wound Study:

Though it was observed that the animals of the *Treated* groups healed faster than the *Control* group, there was no external indication of any major statistical differences between the three groups. However histopathology reports of the skin samples of the 15^{th} post-operative day of the control group, showed a comparatively thin epithelium covering the surface, while in the groups treated with Fibrin and the Fibrin-g-P(HEMA) , the surface was completely bridged by epidermal cells and there was extensive proliferation of the connective and reparative cells over the wounded area.

Thus the animal study clearly showed enhanced wound-healing with Fib- g-P(HEMA) .

CHAPTER VI: SUMMARY AND CONCLUSIONS

DGB was processed from industrial wastes and characterized by analytical methods. It was found to have close similarity with synthetic hydroxyapatite, both in chemical composition and crystal structure. DGB was grafted with PMMA and the graft was characterized. The efficacy of the graft as a bioactive-filler in bone-defects was proved.

FIBRIN was processed from the slaughterhouse waste-blood and grafted with P(HEMA). Fibrin and the grafted biopolymer, Fibrin-g-HEMA were characterized by analytical

methods. The possibility of using this biomaterial for wound-healing was tested on animals and found to enhance the healing process.

It can be concluded that the present work is a useful conglomeration of the natural and synthetic polymers yielding bioactive materials, which can find clinical use in the near future as the DGB-g-PMMA composite proved as a osteo-conductive material with the rabbits and the Fibrin grafted with the bio-compatible P(HEMA), proved bio-active in wound-healing when used on rodents.

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