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Regenerative Medicine- Scaffolds

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Abstract: Ligament tear and its faster healing is a major problem faced by athletes and elderly people who are adversely affected due to its slow regeneration capacity. The striking challenge in bone regeneration is the ability to fabricate synthetic extracellular membrane. To put an end to the slow healing process, Tissue engineering has come up with engineered scaffolds proving faster regeneration process[1,2]. They are agents having an attractive feature to mimic the native environment. Scaffolds are excellent agents in binding the cell to an extracellular matrix of another cell. They help in the multiplication, growth, spread and expansion of the cell and helps in the differentiation of the cells which is required before the regeneration process[3,4]. Fibroblasts which is a type of cell which helps in synthesis of the extracellular matrix is protected by these scaffolds as fibroblasts play a major role in wound healing and also the adult stem cells which are a mass of undifferentiated cells found throughout the body are being protected as they too contribute a major role in healing of damaged tissues[5,6]. In the present study we have prepared scaffolds using biomaterials such as Cellulose, Hydroxyapatite and Graphene Oxide. Scaffolds are being used to induce tissue repair by undamaged cells at the site of injury and provide excellent structural support for the tissue development. The properties of the prepared scaffolds, such as porosity, water adsorption ability and compressive strength are studied. The developed scaffolds were characterized by SEM, FTIR and tensile testing.[7]

Keywords: Bone regeneration, ECM, Fibroblasts, FTIR, SEM.

Introduction

Tissue engineering is an evolving branch providing the need for regeneration of tissues damaged due to injury. The developing field of tissue engineering aims to regenerate damaged tissues by combining cell from the body with highly porous scaffold biomaterials which guide the growth of new tissue[8,9]. An effective method in treating this is the use of scaffolds.

Tissue engineering is an approach for the ligament regeneration that optimizes the response of cell-biomaterial interaction which enhances the faster growth of the damaged tissues[10, 11, 12]. We develop a bone scaffold using bacterial cellulose, hydroxyapatite and bacterial cellulose[13,14,15,16,17]. One of the main advantages of using graphene oxide is its easy dispersability in water and other organic solvents. In this approach we fabricate a bone scaffold with greater thermal stability, Microporosity, greater mechanical strength, decreased water uptake, low degradability, desirable Ca/P ratio, suitable cell viability, capability to conduct as well as induce osteoblast activity. Ideally, the scaffold provides a temporary pathway for regeneration and will degrade either during or after healing. It must be ensured that the degradation products are non-cytotoxic and blood-vessel formation throughout the scaffold is critical to the success of the scaffold[18, 19, 20]. This paper describes the

functional requirements, materials used in developing state of the art of scaffolds for tissue engineering applications.

Materials and Methods

Materials

- Bacterial Cellulose: Acts as 3-D collagen matrix of bone and it will provide higher mechanical strength.
- Hydroxyapatite: It enhances osteoblast growth and will act as an inorganic phase of bone.
- Graphene Oxide: Will increase the surface area of BC and will provide higher mechanical strength

Fabrication of Scaffold

Synthesis of Graphene Oxide nanoparticles is being carried out by Hummers method. Wet precipitation method is used for the formation of hydroxyapatite and also for the combination of Hydroxyapatite (HaP) and graphene oxide (GO). Fabrication of bacterial cellulose, graphene oxide and hydroxyapatite is completed which is being characterized. [21, 22,23,24,35]

Results and discussion

The synthesised and fabricated scaffolds were characterized using XRD, FT Raman, and SEM. And

Cell viability studies for different combinational scaffolds were also been studied using cell lines. [39,40,41,42,43]

FTIR Spectrum

FTIR spectrum of different biomaterials synthesised are as follows.

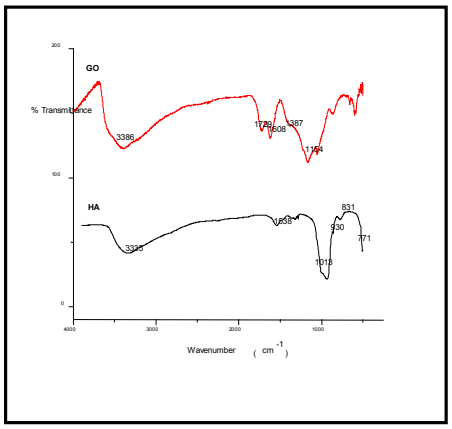


Fig 1: Hydroxyapatite

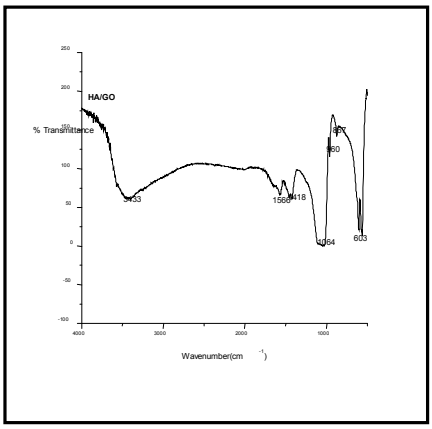


Fig 2: Graphene Oxide

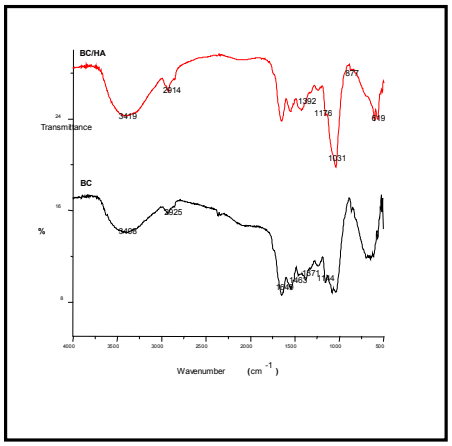


Fig 3: Combination of Bacterial cellulose and Hydroxyapatite

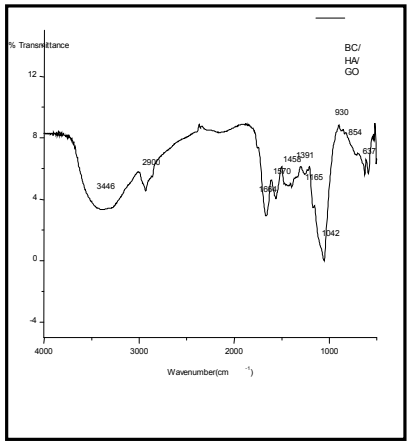


Fig 4: Combination of Bacterial cellulose, HaP and Graphene Oxide

FT RAMAN Spectrum

FT RAMAN analysis was carried out for all the carbonaceous biomaterials and the spectrum is shownbelow.[44,45,46,47,48]

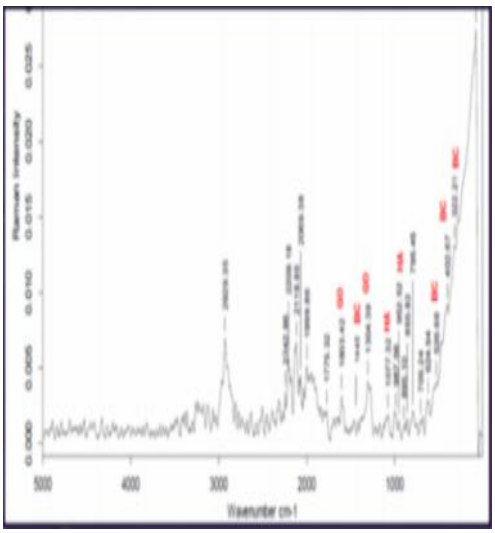


Fig 5: FT RAMAN Spectrum for GO/BC/HaP

From FT RAMAN Spectrum of carbonaceous materials it was noticed that I_d/I_g ratio of GO was 1.3 but generally the ratio would be 0.98. This increase could be due to the presence of HaP between the graphene oxide nanoparticles.

XRD Data

X-ray Diffraction studies for Graphene oxide biomaterial was also examined and the data is as follows.

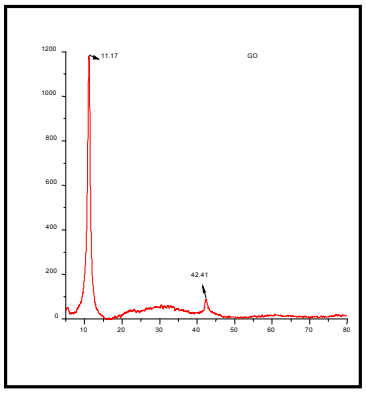


Fig 6: XRD Data of GO BC/HA, BC

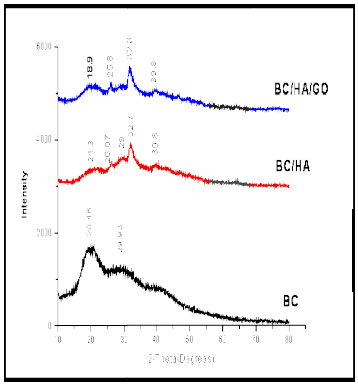


Fig 7: XRD Data for BC/HA/GO,

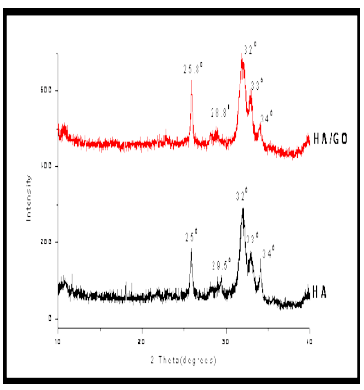


Fig 8: XRD Data for HA/GO, HA

In the XRD of GO [Fig 6], the sharp peak at 11.7^0 is due to the presence of more O_2 functional groups like carboxyl, hydroxyl and epoxy. The peak at 11.17^0C of GO disappeared due to the addition of HA on the surface of O_2 functional groups and the reduction of these groups.

TGA Data

TGA data of different biomaterial, combination of two biomaterials were considered for thermo gravimetric analysis and the data is as follows.

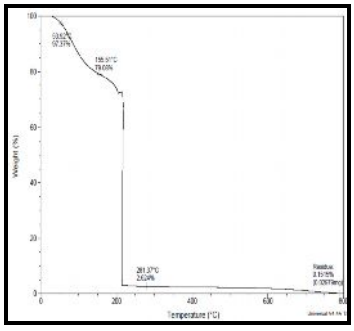


Fig 9: TGA Data of GO

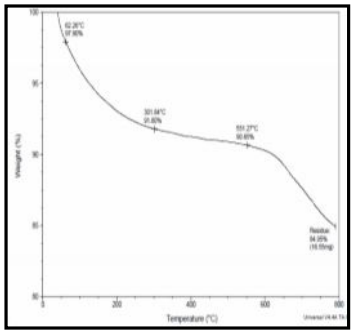


Fig 10: TGA Data of HaP

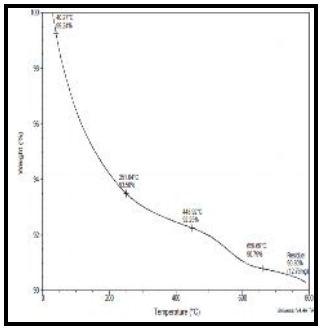


Fig 11: TGA Data of GO/HA

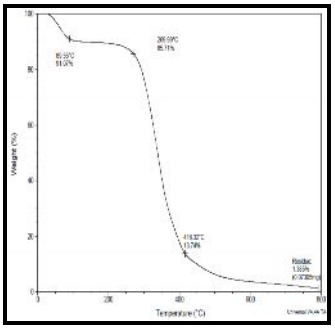


Fig 12: TGA Data of BC

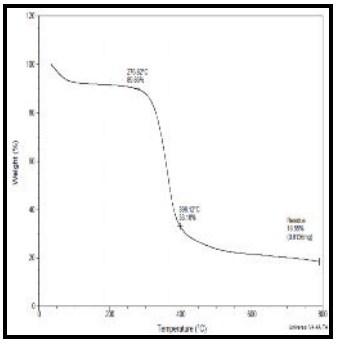


Fig 13: TGA Data for BC/HA

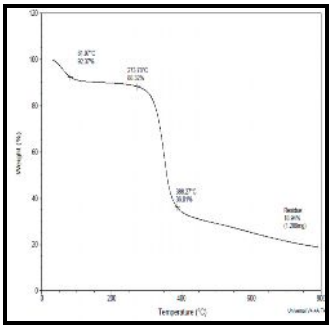


Fig14: TGA Data for BC/HA/GO

SEM Data

The biomaterials were individually viewed under SEM and their Images are as shown below.

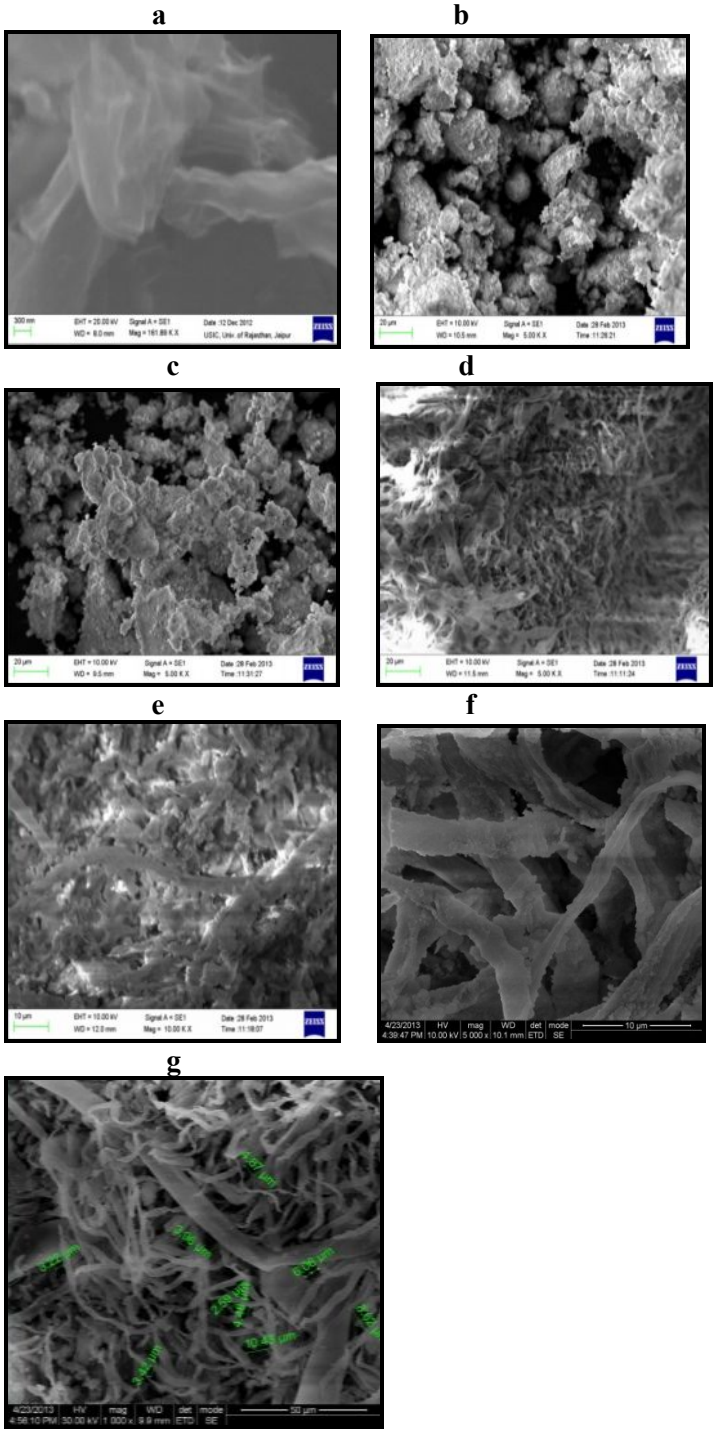


Fig 15: SEM images of (a) GO (b) HA (c) HA/GO (d) BC (e) BC/HA (f,g) BC/HA/GO

SEM EDS and TENSILE STRENGTH[49,50,51,52,53,54]

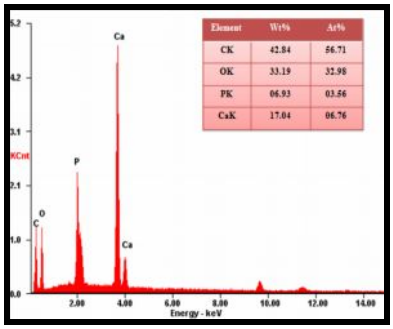


Fig 16: SEM EDS of BC/HA/GO

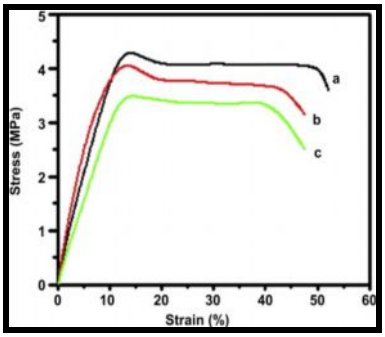


Fig 17: Tensile Strength analysis of (a) BC/HA/GO (b) the BC/HA (c) BC which shows highest peak for bacterial Cellulose Hydroxyapatetite/ graphene oxide

Water Uptake Ratio and *In Vitro* degradation

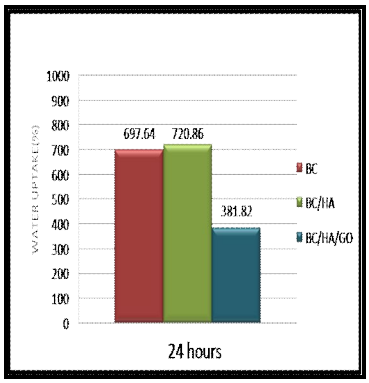


Fig 18: The water uptake ratio was found to be more for the combination of bacterial cellulose/ hydroxyapateite after 24 hours.

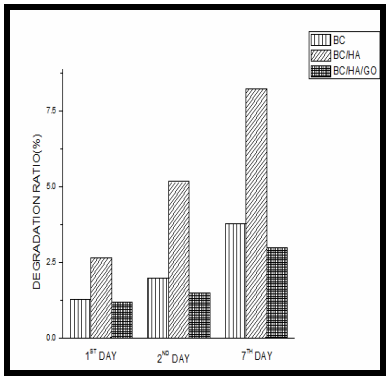
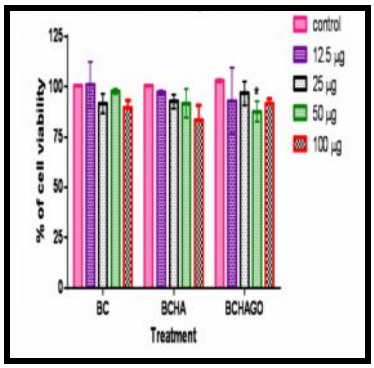


Fig 19: In vitro degradation results for day , day2 and day 7 which shows the increasing concenteration of BC, BC/HA BC/HA/GO

Cell Viability Assay[55,56,57]

a b



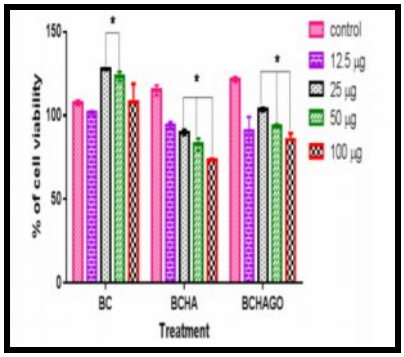


Fig 20(a): Cell viability assay has been done on day 1 and day 4 fig 20(b) which shows the increasing concenteration of bacterial cellulose, bacterial cellulose/hydroxyapatetite, bacterial cellulose, hydroxyapatetie/ graphene oxide.

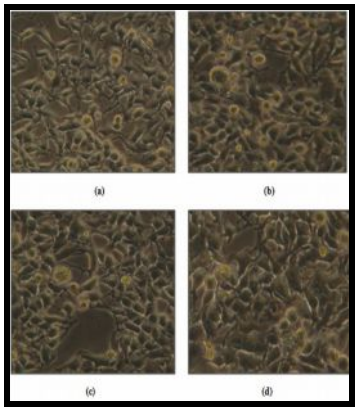


Fig 21: Microscopic images of the viable cells in (a) control cells (b) 100 µg BC, (c) 100 µg BC/HA, (d) 100 µg BC/HA/GO on 4th day.

Conclusion

A novel bone scaffold is fabricated using hydroxyapatite, graphene oxide and bacterial cellulose. When compared this novel scaffold with already existing bone scaffolds with some similar compounds(except graphene oxide) by various physicochemical characterization techniques[58,59,60]. *In vitro* Studies was carried out using MG-63 cell lines which proved that Graphene Oxide at higher concenteration was not cytotoxic and Bacterial Cellulose enhanced the cell viability on 4th day[61,62]. All the results till now prove that this novel scaffold is superior to other bone scaffolds of similar compounds (except Graphene Oxide). The role of the following compounds was elucidated as Bacterial Cellulose forme the matrix to attach Hydroxyapatite and Graphene Oxide particles. Hydroxyapatite formed the mineral phase. Graphene Oxide increased the thermal stability as well as decreased the water uptake.[63]

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