Abstract

Today in Tissue Engineering has gained prominence in the modern research focussed in the conditions of trauma and injury.The bone-derived-osteoblast when seeded on scaffold material has displayed prominence in the treatment of the osseous medical condition.

A promising route towards bone tissue engineering comprised of materials of the composite which can mimic properties of the extracellular matrix (ECM) components. The process development in designing of the cell-material construct to enable regeneration of bone is in increasing demand for the repair of defective and/or diseased bone tissue. With properties of constituents being attributed to both origin and chemical modifications, natural polymer-based, ‘polypeptide-polymer-ceramic-cell material’ shows significant role in bone tissue engineering.

In this study, ‘polypeptide-polymer-ceramic’ scaffold material was successfully designed by alternating freeze-drying and leaching cycle in a 24-well cell culture plates. Lyophilised bioscaffolds were prepared by the addition of amorphous chitosan to hydroxyapatite dispersed in gelatin matrix to evaluate its supportive function in osteoblast regeneration followed by ECM production, in living cells (*in vivo*).

The scaffold materials made from ‘gelatine and/or hydroxyapatite infused with chitosan’ was extensively evaluated for innate properties and morphology in terms of surface and core, crystallinity, physico-chemical, thermo-mechanical, degradation-mineralization measurements.

The innate antibacterial property was assessed to prevent surgical-site infection after scaffold implantation. The presence of all the three raw material in the scaffold favoured

maximum range of antibacterial activities. The contact surface properties are prime factors driving the behaviour of cell immediately after seeding in the composites. They were studied for the optimised surface roughness as suitable for cell attachment and proliferation via AFM. Chitosan inclusion lead to increase in the surface roughness. The uniformity in the interconnected porous architecture facilitating cellular penetration was studied from SEM and TEM. Chitosan introduction resulted in change in scaffold morphology from circular to elliptical. Here, tissue engineering ventures primarily on site-specific architecture following manipulation of cell and matrix. They were characterized for their calcium to phosphate (Ca/P) ratio of the mineral deposition by SEM equipped EDX. EDX based elemental mapping defined the inorganic content in these composites which dropped after fabrication w.r.t the raw materials.

Uni-axial Compressive Strength (UCS) was tested for defining the load-bearing strength of each of the scaffolds via Texture Analyser with 9.81N load cell. Chitosan addition had lead to decrease in flexibility of the scaffolds. The balance between the UCS with the porous architecture was necessary to mimic the ECM function of the Bone to guide cells to grow into the desired physical form and further favour ECM deposition. Moreover, the relative concentration of the polymer-ceramic defined the quality and shelf life of the scaffold in terms of their thermal measurement done by DSC.

The qualitative to quantitative phase development in terms of the innate mineral deposition capability was studied using XRD and FTIR, respectively. The crystal spots determined from SAED pattern of these scaffold materials supported the insights from the physicochemical measurements. Both XRD and SAED demonstrated decrease in crystallinity with increase in gelatine component in the scaffold.

The *in vitro* bio-degradation and bio-mineralization of designed scaffolds was determined by the creation of artificial conditions after immersing them in lysozyme and SBF,

respectively. Broadly, significantly higher degradation and mass increase was observed with the absence of the inorganic component, hydroxyapatite. The slower degradation of the scaffolds enabled it to be retained for longer duration under the culture conditions. Linear and complete degradation of CG omitted it for considerations for cell studies. Gelatin inclusion permitted creation of nucleation sites promoting bioactivity of GH. These results demonstrating the relative behavior of the biomaterial enable the selection of the best cell-biomaterial co-culture system for further bone bioengineering studies.

In vitro osteogenesis of the primary cell line of osteoblasts from both rabbit and human origin were isolated from the bone tissue (rT) and MSC-derived from bone marrow (rM) on gelatin/hydroxylapatite/chitosan based scaffold materials were investigated after the evaluated scaffolds were selected for cell seeding. Out of the evaluated scaffolds, the GH and CGH scaffold materials which displayed maximum resemblance in properties with the natural bone ECM were selected for further *in vitro* osteoblast cell studies.This study has the Central Animal Ethical Committee of Banaras Hindu University (BHU).

After the isolation of both the cell types from the respective cell sources, the counted cells were subcultured in a 24-well cell culture plates and 'Passage-4' was used for the *in vitro* studies done till 'culture day' 28^{th} following the growth curve of the cells. Flow cytometry (FC) classified the isolated MSCs and the differentiated cells distinctively by both positive and negative selection using a panel of surface markers analysed using Fluorescein Iso Thyo Cyanate (FITC) beside the surface attachment study. FC analysis of the cell cycle was done to define the potential proliferating cell population selection for this study.

Again the scaffolds were characterised for altered improved properties with respect to the natural bone after seeding them with cells from rabbit after culture day 21^{st} samples. Surface and core morphology of the 'cell-biomaterial conjugates' was studied from AFM

and SEM-EDX, respectively. CGH displayed denser surface roughness favouring cell attachment indicating prominent role of chitosan in increasing roughness over relatively smoother surface favouring proliferation and calcium adsorption in the protein matrix of the GH scaffold. The chitosan induced surface treatment too lead to increase in the bioactivity in addition to existing hydroxyapatite. It was widely accepted that the bone cell responds to multidimensional cues resulting in the formation of clusters and aggregates, as observed from SEM of rTGH and rTCGH.

The capacity to increase the innate mineral deposition capability of both the types of osteoblast was evaluated onto the scaffolds by physic-chemical characterization. The intensity of minerals deposited by both the cell types on both the scaffold types was distinctively comparable in the X-ray Diffractogram. rTCGH displayed highest deposition in the matrix. FTIR studies the inorganic-part of the deposited mineral was observed to resemble the inorganic-hydroxyapatite in terms of intensities observed after cell-scaffold interaction. The shelf-life of the cell-scaffold conjugate was determined from the DSC scan.

The *in vitro* metabolic behaviour of viable osteoblasts and their growth was studied in replicates (n=5) by Fluorescence Alamar Blue (AB) assay and DNA content using Hoechst 33258. Early differentiation marker Alkaline Phosphatase (ALP) activity was done to determine the potency and Calcium's relative content by Alizarin Red S (ARS) mineralization assay. Using co-culture with CGH, rT over rM showed that rabbit bone extracellular matrix (ECM) mimicking the properties both in *in vitro* studies and scaffold materials micro architecture. The osteogenic nature of the population studied in the scaffolds was assessed from the gene expression of collagenase type-1, osteocalcin and BMP-2 w.r.t GAPDH as internal control by Q-PCR. The expression was subsequently quantified by immunoblot. The subsequent *in vitro* study revealed that among the

biomaterials, ‘chitosan in hydroxyapatite-coated gelatin matrix’ had prominence over ‘gelatin-hydroxyapatite’ only after seeding with rT.

Result show that the cell viability of above 90% was tunable with the seeded cell’s ECM production by the bioactive biomaterial. These rabbit iliac crest-cell- seeded biomaterial (rTCGH) were observed to be appropriate for use in bone bioengineering applications.

A novel combination of scaffold materials-cell interaction was observed when rT was co-cultured with CGH and proved effective in osteogenesis with reference to bone Bioengineering.

Herein, the human-origin bone cells (rT) were seeded in the CGH scaffold types to investigate the best co-culture system. Further, the human bone cell-seeded composite was tested by flow cytometry, CLSM, SEM and DSC.

This study statistically defined and proved that the human bone tissue (hT) derived bone cell seeded in CGH as potential material for minor to severe bone related tissue regenerative studies.