Strontium and selenium doped bioceramics incorporated polyacrylamide-carboxymethylcellulose hydrogel scaffolds: mimicking key features of bone regeneration

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\textbf{ABSTRACT}

Polyacrylamide-carboxymethylcellulose hydrogels lack mechanical strength, uncontrolled rate of degradation, and poor osteogenic properties which can be improved by the incorporation of bioceramics. Strontium and selenium doped bioceramics \(\text{xSrO}\(\text{3-xCaO}\text{4SiO}_2\)(12-y)P\text{2}O\text{5}ySeO\text{2}\y\text{5MgO}\) (where \(x = 6\) mol\%, \(y = 3\) mol\%) incorporated polyacrylamide-carboxymethylcellulose hydrogels were synthesized by free-radical polymerization. Above mentioned bioceramics were prepared by the sol-gel route. XRD analysis indicated an increase in crystallinity with the increment of bioceramics content. With the incorporation of bioceramics, the apatite phase formation was observed on 1\textsuperscript{st} day of immersion in simulated body fluid at pH 7.4. Strontium and selenium doped bioceramics impregnated hydrogels exhibited an inhibitory effect on human osteosarcoma MG63 cell line. Moreover, bioceramics incorporated hydrogels provide a conducive environment for MC3T3-E1 osteoblast cell line proliferation, adhesion and also exhibited good alkaline phosphatase activity. Synthesized hydrogels indicated special osteogenic properties and can be potentially utilized in the hard tissue regeneration as well as for recovery of bone cancer patients.

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1. Introduction

Bone tissue regeneration is one of the most prominent medical research areas looking for solutions due to widespread people carrying bone defects and disorders. In this modern era, the increase in the alarming rates of localized bone and tissue disorders, osteoporosis, congenital defects, traumatic injuries, and organ failure make an urgent call for tissue engineering. Tissue engineering revolutionizes the traditional approach of bone grafting. There are various drawbacks associated with grafts, such as donor morbidity, the transmission of disease, and limited availability. To overcome these problems, tremendous efforts for bone tissue repair strategies have been undertaken to develop promising synthetic scaffolds which mimic the body tissue environment and these must be bi-functional, porous, and should be able to provide osteoblast cell adhesion. The idea of designing an implant material for successful implication in the orthopedic field has always been a mystery but the emerging bone tissue regeneration field brings global developments in this area [1,2].

In recent times, polymeric hydrogels have become promising biomaterials for their usage in biomedical applications. Hydrogels provide a suitable microenvironment for cell adhesion, proliferation, migration, and differentiation in repairing and restoration of the damaged tissue structure. Hydrogels can imbibe a large amount of water in a swollen state which makes them similar to living tissues and therefore, they may easily shape and reshape during tissue formation after implantation. However, natural polymeric hydrogels are soft, fragile, and lack mechanical strength, which restricts their utility as a scaffold material for tissue engineering. On the other hand, incorporation of synthetic biomaterials to the natural polymer matrix improves the mechanical performance and flexibility and hence, bioceramics incorporated hydrogels may act as a desirable candidate for soft and hard tissue engineering applications [3–6]. In the light of this situation, authors have chosen the study of bioceramics incorporated hydrogels for bone regeneration applications.

Carboxymethylcellulose (CMC) is the major cellulose derivative and water-soluble cellulose ether anionic polysaccharide having carboxylic groups which form strong coordination with metal ions. CMC shows good biocompatibility, biodegradability, nontoxicity and it is widely used in various tissue engineering, wound dressing, and drug delivery applications [7–9]. Acrylamide (AAm) is the monomer which polymerizes to form
polyacrylamide (PAAm) as a synthetic hydrophilic polymer and exhibit very poor mechanical strength due to its soft and brittle nature [10]. PAAm has been used in diverse applications in the biomedical field, such as tissue engineering, drug delivery, and biosensor fluid. However, the utility of these polymeric scaffolds in tissue engineering has been restricted due to their release of acidic byproducts upon degradation of polymers on exposure with the physiological fluid as simulated body fluid (SBF) which adversely affects the cell viability and their proliferation. Recent studies demonstrated that the incorporation of bioceramics into the composite polymer matrix reduces the accumulation of acidic byproducts and provides a conducive environment for cell growth.

Bioceramics on exposure with the body fluid initiate the formation of hydroxyapatite (HA) which mimics the composition of natural bone and promotes osteoconductivity, bioactivity, osteoinductivity, and also enhances the mechanical properties [11–14]. From the biological aspect, elements such as calcium (Ca), phosphorus (P), silica(Si), magnesium (Mg), strontium (Sr) and selenium(Se) ions are the important trace metals in the body that promote and aid in bone growth. The concept of incorporation of these trace metals in the bioceramics system is new which helps to improve the rate and mechanism of bone formation, osteoblastic proliferation, and bioactivity. For instance, calcium and phosphorus are major ions of the inorganic phase of bone and stimulates osteoblast proliferation, whereas silica ions promote osteoinduction and osteogenesis. Mg is associated with biominalization and control mineral metabolism and also, improves the mechanical properties of sol-gel synthesized biomaterials. Further, Sr doped bioactive systems are used for the treatment of osteoporosis. They increase the rate of formation of new bone and inhibit the osteoclast activity of bone resorption and also intend to improve bone remodeling. Se is also another important element that is found in a very minimal amount in the human body but has paramount significance due to antioxidant, antibacterial and anticancer properties. Therefore, Se deficiency slows down the rate of bone formation and also, even leads to the development of Parkinson’s disease in long run [15,16]. In the current scenario, polymer-bioceramics composite scaffold holds paramount importance in the field of tissue engineering. For example, Pasqui.et.al [17] have inserted HA nanocrystals within the hybrid CMC-based hydrogels and showed enhanced MG63 cell viability as compared to CMC hydrogels without HA. In another study, Fang.et.al [18] have developed the HA mineralized PAAm/Dextran hydrogel that exhibited increased mechanical strength and excellent osteointegration. Li et al. [19] have fabricated in situ formation of HA in tough PAAm hydrogels that resulted in enhanced mechanical properties and osteoblast adhesion. In another study, Keenan.et.al [20] have prepared gallium doped bioactive glass (0.425SiO₂-0.10Na₂O-0.08CaO-(0.40–x)ZnO-(x)Ga₂O₃) into CMC–dextran hydrogels which provided the conducive environment for L-929 fibroblast and MC3T3-E1 osteoblast viability. In the light of this situation, it can be inferred that PAAm/CMC (PCMC) polymeric-network may provide a better hydrogel-system when incorporated with bioceramics for bone tissue engineering applications.

In the presented work, authors report about the development of hydrogels composed of PAAm/CMC by varying amounts of sol-gel derived strontium and selenium doped-bioceramics (SSBC) such as 0, 10, 20, and 30 wt% (of polymeric content). The synthesized freeze-dried hydrogels have exhibited very soft and gel-like behavior on the absorption of water due to the hydrophilic nature of polymers. The properties of composite hydrogels, including mechanical properties, in vitro biominalization and the cellular response, were analyzed. The obtained hydrogels were characterized structurally by X-ray diffraction and Fourier transform infrared spectroscopy techniques. Further, to analyze the surface morphology of freeze-dried hydrogels, scanning electron microscopy was used. Zeta potential was performed to determine the surface charge of the hydrogels. Also, compressive strength, in vitro degradation, swelling percentage, and porosity of the synthesized hydrogels were explored. The main objectives of the studies include; 1) To determine the apatite formation 2) To assess the in-vitro anticancerous behavior of samples.

2. Material and methods

The chemicals used for the synthesis of the bioceramics were tetraethylorthosilicate (TEOS), triethyl phosphate (TEP), sodium selenate pentahydrate, strontium nitrate, magnesium nitrate hexahydrate, and calcium nitrate tetrahydrate obtained from Merck (AR grade). The same were used as precursors of silicon dioxide, phosphorus pentaoxide, selenium trioxide, strontium oxide, magnesium oxide, and calcium oxide. 1 M nitric acid solution was used as a catalyst for hydrolysis. The chemicals used for the synthesis of hydrogels are Acrylamide (AAM) as a monomer (polymerized to form PAAm), carboxymethylcellulose (CMC) as the cellulose ether anionic polysaccharide, ammonium persulfate (APS) as an initiator, and N,N′-methylenebisacrylamide (MBA) as the crosslinker obtained from Sigma Aldrich, India.

2.1. Preparation of bioceramics

Bioceramic composition

$\text{xSrO.} (43-x)\text{CaO.} 40\text{SiO}_2. (12-y)\text{P}_2\text{O}_5. y\text{SeO}_3. 5\text{MgO}$ (where $x = 6$ mol%, $y = 3$ mol%) was prepared by sol-gel method according to our previous study [21]. In brief, TEOS was added in 1 M HNO₃ solution (TEOS and water
molar ratio equal to eight) and the solution was kept for stirring for 1 h for complete hydrolysis. TEP, calcium nitrate tetrahydrate, strontium nitrate, sodium selenite pentahydrate, and magnesium nitrate hexahydrate were dissolved in 1 M HNO₃ for more than 0.5 h. Both solutions were mixed and kept under vigorous stirring. After 1 h of vigorous stirring, a transparent sol was obtained. The transparent sol was aged in an air-tight beaker for 3 days, which was converted into a gel. The gel was calcinated by drying at 700°C for 6 h and ground into a fine powder using conventional ceramic mortar and pestle.

2.2. Synthesis of PCMC and PCMC/SSBC hydrogel scaffolds

For hydrogel preparation (see Figure 1), the sieved bioceramics samples (10, 20, and 30 wt% of total polymeric content) were first dispersed in distilled water and kept for mechanical stirring in distilled water (50 mL) for 1 h and then 5.0 g of AAm and 0.5 g of CMC were added into prepared bioceramics suspensions. Further, MBA (0.04 g) and APS (0.03 g) were added and dissolved under mechanical stirring for 1 h to get a homogenous suspension solution. These prepared final solutions were poured in 24 well plates (i.e., mold) and kept in the oven at 60°C for 6 h to facilitate free-radical polymerization. Polymerized hydrogels were washed with distilled water to remove the unreacted monomers and reagents. Finally, some polymerized hydrogels were kept in a refrigerator. This process was further followed by freeze-drying at -40°C for 6 days to remove the solvent (distilled water) from the samples. Similarly, 5.0 g of AAm and 0.5 g of CMC was dissolved in distilled water without bioceramics and polymerized using the same procedure as described above. The polymerized hydrogels were designated as PCMC/SSBC-0, PCMC/SSBC-10, PCMC/SSBC-20, and PCMC/SSBC-30 for PAAm/CMC hydrogel and PAAm/CMC hydrogels with 10, 20, and 30 wt% of strontium and selenium doped bioceramics (SSBC) of total polymeric amount. Details are provided in Table 1.

2.3. Characterization techniques

2.3.1. X-ray diffraction (XRD) studies or crystallinity studies

XRD of the prepared samples was undertaken to determine the phase composition and crystalline behavior of the samples by using Bruker D8 focus X-Ray diffractometer at a scanning rate of 2° per minute in 2θ range of 10–60° at the step size of 0.02° s⁻¹. The presence of crystalline phases was established by using JCPDS files in JCPDS software. XRD data have been plotted by using origin software 8.5.1 and the data were interpreted.

2.3.2. Fourier transform infrared (FTIR) spectroscopy

The characteristic functional groups and chemical bonds present in the PCMC/SSBC hydrogel scaffolds were investigated by Fourier Transform Infrared spectroscopy in transmittance mode from 3500 to 450 cm⁻¹ by using Perkin Elmer spectrometer (C92035), Germany.

Figure 1. Schematic of synthesis technique of the bioceramics incorporated hydrogels.
2.3.3. Scanning electron microscopy (SEM)
The surface morphology of the hydrogels before and after the treatment with SBF was determined by SEM JEOL/EO (version 1.0), at SAI Labs (Sophisticated Analytical Instrumental Laboratory), Thapar University, India. Before the analysis, hydrogels were washed with acetone and DI water and dried in an oven at 37°C. Further, a gold sputter coating was performed to make samples conductive.

2.3.4. In-vitro biomineralization
The in vitro bioactivity of the prepared hydrogels was evaluated by their ability to form an apatite layer after soaking them in simulated body fluid (SBF) solution for 1, 3, 7, and 14 days. SBF was prepared by the protocol reported by Kokubo et al. [22]. 100 mg of the sample was kept in 50 mL SBF solution in an incubator at 37°C. To maintain the accurate consistency of ions, the medium was renewed every 12 h by replacing 20 mL of medium with the fresh medium. After being removed from SBF, samples were rinsed with distilled water and allowed to dry at 37°C.

2.3.5. Swelling ratio (%)
The swelling behavior of synthesized hydrogel scaffolds was evaluated by immersion in phosphate buffer saline solution (PBS pH-7.4) at 37°C for 60 h. In this experiment, dried scaffolds (Wᵢ) were placed in PBS solution and taken-out after different time intervals, i.e. 10, 20, 30, 40, 50, and 60 h. Excess water was removed by filter paper and wet weight (Wᵢ) is recorded. The amount of water absorbed in the hydrogel scaffolds was calculated by using the relation:

\[
\text{Swelling Ratio}\% = \frac{W_f - W_i}{W_i} \times 100
\]

2.3.6. In-vitro degradation studies
For in-vitro degradation analysis, 200 mg of hydrogel was immersed in 20 mL of phosphate buffer saline (pH value =7.4) at 37°C for 3, 7, 14, and 28 days without the replacement of the solution. The immersed hydrogels were removed from the solution, washed and dried in the oven at 37°C for 48 h. The final mass loss% of samples was calculated as follows:

\[
\text{Mass Loss}\% = \frac{M_a - M_b}{M_b} \times 100
\]

Where \(M_a\) = initial weight of hydrogel before immersion in PBS and \(M_b\) = final weight of hydrogel after degradation.

2.3.7. Determination of porosity of hydrogels
The porosity of the hydrogels was determined by the liquid displacement method [23]. The composite hydrogels were immersed in the known volume of the ethanol, i.e., 20 mL (chosen as the displacement liquid) for 48 h and weight was measured for regular intervals until the weight becomes constant to acquire saturation. Then, samples were weighed after they were dried with the filter paper to remove the excess ethanol. The porosity of composite hydrogels was calculated by using the following equation

\[
\text{Porosity}(\%) = \frac{V_1 - V_2}{V_2 - V_3} \times 100
\]

Where \(V_1\) is the volume of ethanol, \(V_2\) is the volume of ethanol and ethanol immersed hydrogels, and \(V_3\) is the volume of remaining ethanol when the immersed hydrogel has been removed.

2.3.8. Compression analysis studies
Compressive properties of hydrogels in the wet state were analyzed using dynamic mechanical analysis (Q800 DMA; TA Instruments) in the compression mode with a preload force of 0.05 N at 37°C (body temperature) in a constant temperature environment at a rate of 0.5 N/min. Cylindrical-shaped wet hydrogels with diameters and heights ranging from 10 to 12 mm and 6 to 8 mm, respectively, were used to generate stress–strain curves [24].

2.3.9. In-vitro antiproliferative activity
MG-63 human osteoblast-like cells were procured from NCCS Pune, India, and cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (Biological Systems) and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). MG-63 cells were seeded at the concentration of 5 × 10³ per well in a sterilized 96 well plate. Cell viability was tested by an indirect method. Briefly, the 300 mg of hydrogels were dissolved in 10 mL of DMEM medium and incubated at 37°C overnight. The medium was collected and filtered with a 0.22 µm filter to assure sterility. After 1 day of cell seeding, they were incubated with a filtered medium isolated from the polymer for 48 h. The MTT (3-[4,5-di-methylthiazol-2-yl]-

Table 1. Composition of hydrogel scaffolds.

| Sample       | AAm (g) | CMC (g) | MBA (g) | APS (g) | SSBC (wt%) | Water (mL) |
|--------------|---------|---------|---------|---------|------------|------------|
| PCMC/SSBC-0  | 5       | 0.5     | 0.04    | 0.03    | 0          | 50         |
| PCMC/SSBC-10 | 5       | 0.5     | 0.04    | 0.03    | 10         | 50         |
| PCMC/SSBC-20 | 5       | 0.5     | 0.04    | 0.03    | 20         | 50         |
| PCMC/SSBC-30 | 5       | 0.5     | 0.04    | 0.03    | 30         | 50         |
2,5-diphenyl tetrazolium bromide) dye has been used to check the cell integrity. The MG-63 cells were incubated with the sample concentration (5 mg/mL) in 96 well culture plates for 72 h. Tissue culture grade cover-slip (Himedia) was used as a positive control for comparison. Each sample was tested in triplicate. Afterward, the MG-63 cells were incubated for 72 h incubation. 500 μL MTT was added in a non-serum medium which leads to the formation of purple color. The numbers of living cells were quantified spectrophotometrically with 570 nm UV radiation by using Labsystem Multiskan EX ELISA reader.

2.3.10. Annexin V assay
MG63 cells were seeded in a six-well plate at a density of 1 × 10⁵ cells/well and cultured with DMEM medium for 24 h. Subsequently, these cells were treated with bioceramics doped hydrogel and pure hydrogel for 24 h. After the incubation period, the cell pellets were collected by removing the DMEM medium and then washed with cold PBS twice. After this, cell pellets were resuspended in a 1 mL binding buffer. Further, 5 μL of Annexin-V FITC (Sigma-Aldrich) conjugate was added along with 5 μL of propidium iodide for 15 minutes in the dark. Annexin V stain was used along with propidium iodide (PI) to identify the MG63 cells in early and late apoptosis. PI enters the damaged and dead cells but it was unable to permeate cells with the intact membranes. A negative PI and Annexin V result revealed that the cells were viable, whereas a positive Annexin V and negative PI results suggest early apoptosis. A positive Annexin V and PI result indicates that the cells can be considered dead or in late apoptosis [25]. Finally, these cells were analyzed by BD Accuri Software using the flow cytometry (BD Accuri C6 Flow Cytometer, BD Biosciences).

2.3.11. In-vitro cytocompatibility studies
Sterilized cylindrical hybrid hydrogels (by soaking in 70% ethanol for 1 day and further washed with PBS (3 X) and exposed to UV light for 2 h) were soaked in culture medium (DMEM with 10% FBS and 1% penicillin G-streptomycin) for 4 h and seeded with 2 mL of MC3T3-E1 osteoblastic cells (5 × 10⁴ cells/cm²). The cell-seeded hybrid hydrogels (15 mg) were incubated with 5% CO₂ at 37 °C for 1 or 3 days. Each sample was tested in triplicate (n = 3) using a 24-well plate [26].

2.3.11.1. Cell viability and proliferations. Cell proliferation was evaluated by staining MC3T3-E1 cells with MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium) for 1 and 3 days of incubation. An MTS solution (CellTiter 96® AQuous One Solution Cell Proliferation Assay, Promega) was assayed according to the manufacturer’s instructions. Each well was added with 100 μL of fresh DMEM medium and 20 μL of reagent was added to each well and incubated with 5% CO₂ at 37 °C for 3 h. After incubation, the samples were then transferred to the 96-well plate. The absorbance of the obtained solution was recorded at a wavelength of 490 nm using a microplate reader (Epoch Microplate Spectrophotometer, BioTek Instrument, USA). The supernatant was removed, washed thrice with PBS, and then fixed with an aqueous 2.5% Glutaraldehyde solution for 15 min. Lastly, the desired hybrid hydrogels were dehydrated and freeze-dried; the surface morphology (cell adhesion and growth) was subsequently evaluated via FESEM (FEI Nova NANOSEM 650, Australia). Hybrid hydrogel samples that were incubated (Days 1 and 3) in MC3T3-E1 cells were freeze-dried (−80 °C) and were coated with a fine layer of platinum at a low deposition rate using an ion sputter coater.

2.3.11.2. Cytoskeletal studies. Since the MC3T3-E1 cells were on an opaque solution, they can be viewed in an inverted microscope. 500 μL of 3.7% formaldehyde in 2-(N-morpholino)ethane sulfonic acid (MES) buffer was added to each well with test specimen (hydrogel) and tissue culture plastic, i.e., control wells to fix the cells. These cells were incubated for at least 10 min. Formaldehyde solution was removed and the samples were washed with PBS. 500 μL of 0.5% TritonX100 in MES buffer was added for 3–5 min to permeabilized the fixed cells for staining with the compound. TritonX100 was removed and samples were washed with PBS. Add 200 μL Alexa Fluor 488 phalloidin (Invitrogen, Oregon) stains to each sample and incubate for 20 min, covered and protected from light (Preparation of stain stock (4 mL): 20 μL Alexa Fluor 488 in 3980 μL of PBS). After 20 min incubation with stain, samples were removed from wells using forceps and inverted into a large glass slide. After the examination on the inverted microscope using mercury lamp light source Nikon Eclipse TE300 microscope 10X, DAPI (4,6-diamidino- 2-phenylindole) is the blue-fluorescent DNA stain and GFP are a green fluorescent protein and both DAPI and GFP images were recorded [27,28].

2.3.11.3. Alkaline phosphatase (ALP) activity assay. The MC3T3-E1 cells were cultured using the above procedure within hydrogel samples and at 7, 10, and 14 days the supernatant was collected from all wells and centrifuged to remove particulates, if any. Aliquots of the supernatants were dispensed into Eppendorf tubes for storage at −70°C and assayed for Alkaline Phosphatase Activity (QuantiChrom™ Alkaline Phosphatase Assay Kit -DALP-250, Bio Assay systems, USA). The protocol as per the instructional manual was performed [29].
2.4. Statistical analysis

Data from at least three independent experiments are expressed as mean ± SD (standard deviation), indicating error bars. Statistical significance was calculated using a one-way ANOVA test, followed by the Turkey–Kramer post hoc test (SPSS16 software; Chicago, IL, USA). The probability (P), P<0.05 was considered statistically significant.

3. Results and discussion

3.1. Evaluation of successful incorporation of bioceramics into hydrogels

The strontium and selenium doped bioceramics have gained special interest due to the significant curative potential of these ions in bone repair and regeneration. The authors have selected the molar ratio of strontium twice the molar ratio of selenium in the bioceramics composition which can balance the toxicity of the selenium. Also, bioceramics synthesized by sol-gel technique led to the enhancement of the surface area, chemical reactivity, and rate of the apatite formation. The strontium and selenium doped bioceramics incorporated hydrogels can be used for the treatment of bone-related disorders as strontium is the element that promotes osteoblast adhesion and proliferation whereas selenium is the strong anticanerous agent and used for the treatment of bone cancer patients. The successful incorporation of the bioceramics into hydrogels is depicted from the analysis of XRD, FTIR, and SEM data.

3.1.1. Before in vitro analysis

Figure 2 (a-d) illustrates the XRD spectra of pure polymer hydrogel scaffolds (PCMC/SSBC-0) and bioceramics incorporated polymer hydrogel scaffolds (PCMC/SSBC-10, PCMC/SSBC-20 and PCMC/SSBC-30). In PCMC hydrogel, the characteristic peaks at 14.5°, 17.58° and 22.3° represent the successful formation of PAAm and the typical characteristic diffraction peak of CMC positioned around 20.7° showed the homogenous incorporation and entanglement of CMC and PAAm polymeric chains. Also, in PCMC/SSBC hydrogels, the peak of whitlockite (JCPDS Card number 70–2064) at 31.3° was additionally observed for bioceramics samples. PCMC/SSBC-0 hydrogel showed non-crystalline diffraction peaks.
Figure 3. FTIR spectra of hydrogel scaffolds before and after in vitro analysis for 1, 3, 7 and 14 days.

Figure 4. Crosslinking of Carboxymethylcellulose (CMC), Acrylamide (PAAm) and strontium/selenium doped bioceramics.
get deposited and led to the formation of apatite. Moreover, H⁺ ions from CMC also take initiative in breaking the silica bridging oxygen bonds (Si-O-Si) and led to the formation of silanol groups (SiOH) which act as nucleation sites for the apatite formation.

XRD study demonstrates the initiation of apatite formation takes place after 7 and 14 days of immersion in SBF as indicated by the very low-intensity peaks of HA (JCPDS Card number 72–1243) at around 31.7° and 46.7° for the hydrogel PCMC/SSBC-0 (Figure 2a). Further, the apatite-forming ability was enhanced with the addition of SSBC to the PCMC hydrogels. The sharp and intense peaks of HA were observed during 1st day for the hydrogels PCMC/SSBC-20 and PCMC/SSBC-30 (Figure 2c–d). The apatite layer formation on hydrogel scaffolds on 1st day of immersion in SBF indicated the good bioactive behavior of scaffolds in vitro and it can be speculated that these scaffolds may easily form the bond with the living tissues and lead to quick recovery and regeneration of damaged tissues at the defect site after implantation in the body. PCMC/SSBC-0 shows more amorphous behavior, whereas the incorporation of SSBC improves the crystalline behavior of PCMC/SSBC-10, PCMC/SSBC-20, and PCMC/SSBC-30 hydrogels. PCMC/SSBC-20 revealed more intense peaks of apatite as compared to PCMC/SSBC-30 which can be related to higher porosity (Table 3.) which results in increased ionic dissolution and rapid ionic exchange with SBF and enhance the apatite formation whereas for PCMC/SSBC-30, incorporated bioceramics fill pores of hydrogels reducing the porosity which lead to the reduce ionic exchange between sample and SBF and results in less intense apatite peaks as compared to PCMC/SSBC-20.

The XRD analysis was also corroborated by the FTIR analysis. The intense characteristic peaks observed at 1032–1026 cm⁻¹, 559 cm⁻¹ and 603 cm⁻¹ are related to different vibration modes of the phosphate (PO₄³⁻) group. Also, the vibrations at 1454 cm⁻¹, 1425 cm⁻¹, and 878 cm⁻¹ correspond to the carbonate (CO₃²⁻) group (see Figure 3a–d)). The existence of these apatite and carbonate peaks indicate the formation of carbonated-HA on the surface of hydrogel [35,36].

3.1.3. **SEM analysis**

SEM micrographs of the hydrogel PCMC/SSBC-20 before and after immersion in SBF for different periods are demonstrated in (Figure 6a–b)). Soaked samples in SBF were filtered out and washed with acetone followed by distilled water and were dried at 45°C. During in vitro analysis, it was observed that the morphology of hydrogel after the immersion for 14 days was slightly different from samples before immersion in SBF. After 14 days, the surface of the hydrogel was observed to be covered completely with apatite growth (Figure 6b). This effect was due to the incorporated bioceramics into hydrogels initiated ionic exchange reaction on exposure with SBF which led to the formation of silanol groups on the surface of the composite hydrogel. The silanol groups provide the nucleation sites for the apatite layer formation. Moreover, the presence of silica groups on the surface of the hydrogel is also confirmed from FTIR spectra. Furthermore, these representative SEM images clearly show the porous microstructures of freeze-dried hydrogels. Many authors speculated that the porous microstructures of the hydrogels were favorable for the vascularization, penetration of essential nutrients, cell adhesion, and proliferation [37,38]. The Energy Dispersive X-ray (EDX) technique was used to evaluate the strontium and selenium in the samples and the at % of Sr and Se are shown in Table 2.
3.2. Swelling ratio

The swelling behavior of PCMC/SSBC-0, PCMC/SSBC-10, PCMC/SSBC-20, and PCMC/SSBC-30 hydrogels was analyzed from their swelling rates in PBS at 37°C, as shown in Figure 7. PAAm is hydrophilic due to its carboxyl amide (CONH₂) group that leads the polymer network to expand so that they easily imbibe the water, whereas the carboxylate ions (-COO⁻) from CMC facilitate more water gulping capacity due to their ionic-repulsion, thereby increasing the voids within the polymeric-network [8,34]. These results were in-line with previous reports. Tulain et al. [39] have reported that the presence of hydrophilic groups – COOH and – CONH₂ leads the polymer network to expand so that they easily imbibe the water, whereas the carboxylate ions (-COO⁻) from CMC facilitate more water gulping capacity due to their ionic-repulsion, thereby increasing the voids within the polymeric-network [8,34]. These results were in-line with previous reports. Tulain et al. [39] have reported that the presence of hydrophilic groups – COOH and – CONH₂ enhances the water sorption capacity at pH 7.4. PCMC/SSBC-0 hydrogel shows the maximum swelling up to 767%, while PCMC/SSBC-10 hydrogel shows a decrease in swelling ratio as compared to PCMC/SSBC-0 which is further decreased in PCMC/SSBC-20 and PCMC/SSBC-30 hydrogels as the SSBC content is increased. This possibly happens due to the high ionic crosslinking between the negatively charged carboxylate group (COO⁻ ions) of CMC and the positive Ca²⁺ ions and other modifier cations, such as Mg²⁺ and Sr²⁺ of the SSBC. Also, these positively charged ions from SSBC neutralize the negatively charged -COO⁻ ion repulsion and reduced the polymer network expansion. This mechanism restricts a large amount of water penetration within the polymer network and thereby, decreases the swelling rate. PCMC/SSBC-30 hydrogel shows the highest decrease in swelling behavior up to 409%. Long et al. [40] reported that the increase in

Table 2. EDX of hydrogel scaffolds.

| Samples       | Sr(Atomic%) | Se(Atomic%) |
|---------------|-------------|-------------|
| PCMC/SSBC-0   | –           | –           |
| PCMC/SSBC-10  | 0.65        | 0.32        |
| PCMC/SSBC-20  | 1.56        | 0.66        |
| PCMC/SSBC-30  | 2.33        | 1.68        |

Table 3. Porosity and mass loss percentage in PBS of synthesized samples.

| Sample       | Porosity (%) | Mass Loss (%) |
|--------------|--------------|---------------|
| PCMC/SSBC-0  | 86           | 18            |
| PCMC/SSBC-10 | 82           | 14            |
| PCMC/SSBC-20 | 80           | 12            |
| PCMC/SSBC-30 | 72           | 9             |

Figure 6. SEM micrographs of PCMC/SSBC-20 before and after immersion in SBF.

Figure 7. Swelling ratio (%) of hydrogel scaffolds in PBS (pH~7.4 at 37°C). Error bars signify the standard deviation (SD) observed for three independent experiments.
water uptake by polymer network decreases the structural and mechanical stability. Therefore, the swelling rate of the scaffolds should be in optimum range or must be tunable for better use of scaffolds in bone healing applications.

### 3.3. In vitro degradation studies

In vitro degradation studies have been considered as an efficient tool to determine the ability of prepared hydrogels to degrade at a similar rate to match the pace of growing tissues. The CMC and PAAm degradation induces the production of acidic byproducts which leads to a decrease in pH and this might result in bone loss, cell apoptosis, and an increase in the vulnerability to local inflammation leading to chronic diseases. On the other hand, the release of alkali ions from bioceramics incorporated in composite hydrogels on exposure with PBS is advantageous since it subsequently converts the acidic pH to basic which is essential for osteoblast proliferation and tissue regeneration [41]. Figure 8 demonstrates the biodegradability of PCMC/SSBC-0, PCMC/SSBC-10, PCMC/SSBC-20, and PCMC/SSBC-30 hydrogel scaffolds in PBS (at pH = 7.4). PCMC/SSBC-0 hydrogel shows maximum mass loss % up to 18% which is due to the presence of highly water-soluble groups, such as -COOH group of CMC and was observed after 1 to 21 days of immersion in PBS. For PCMC/SSBC-10, PCMC/SSBC-20 and PCMC/SSBC-30 hydrogels, the incorporation of strontium and selenium doped bioceramics particles has the different effect on degradation rate. On interaction with PBS, the hydrogels containing SSBC release alkali ions, such as Ca, Si, and other ions present in the SSBC which interact with the -COO⁻ ions of CMC and neutralize the charge by the formation of -COO⁻/Ca²⁺. The counterbalance of charges due to the liberation of alkali ions from the SSBC also leads to a decrease in the degradation rate. Many authors speculated that the natural and slow degradations of hydrogel within an adequate period is highly beneficial for new tissue regeneration, cell attachment, and its differentiation contributes to the successful implantation without any medical inadequacy [42]. The degradation rate starts decreasing with the increasing concentration of bioceramics in the hydrogels as compared to the pure hydrogel. PCMC/SSBC-10 hydrogel shows the decrease in the degradation rate up to 14%, PCMC/SSBC-20 up to 12%, and PCMC/SSBC-30 up to 9% (significantly lower than PCMC/SSBC-0 hydrogel) after 21 days of immersion in PBS. Our results are supported by the analysis of Peter.et.al [43] who reported that the incorporation of bioactive glass ceramics into chitosan hydrogels led to a reduction in the degradation rate in PBS. The controlled degradation rate is beneficial for bone tissue engineering as compared to the rapid rate of degradation.

### 3.4. Evaluation of porosity of hydrogel scaffolds

Porosity is a vital parameter for osteoconduction and tissue engineering. Table 3 presents the porosity of the composite hydrogels (with and without SSBC). The addition of SSBC to the PCMC composite hydrogels leads to the formation of HA which gets deposited over the surface as well as inside the pores and decreases the porosity of the hydrogels. The porosity (%) was observed to be decreased for PCMC/SSBC-0 (~86%), PCMC/SSBC-10 (~82%), PCMC/SSBC-20 (~80%) and PCMC/SSBC-30 (~72%) hydrogels, as the SSBC content was increased in the polymeric matrix. PCMC/SSBC-20 hydrogel showed the maximum apatite formation (see Figure 2c) with the optimum reduced porosity (80%) which is quite suitable to facilitate the penetration of the nutrients and provide a conducive environment for cell adhesion and proliferation. Holtorf et al. [44]. have investigated that the scaffolds with a porosity of 80% facilitate early osteoblast cell differentiation. Further, the

![Figure 8](image-url) (a) Mass loss (%) of hydrogel scaffolds in PBS (pH~7.4 at 37 °C). Error bars signify the standard deviation (SD) observed for three independent experiments.
porosity was reduced with the increase in SSBC content due to the extensive physical and ionic crosslinking between ions (Ca$^{2+}$, Mg$^{2+}$, and Sr$^{2+}$) present in SSBC and the polymer functional group (−COO$^-$) which reduces the carboxylate group ionic repulsion and network expansion. Our results are in agreement with the literature of other bioceramics incorporated hydrogels with reduced porosity [10,13]. PCMC/SSBC-30 exhibited the maximum reduced porosity which results in a decrease in intensity of apatite peaks due to slow ionic dissolution rate (Figure 1d).

### 3.5. DMA compressive testing

The stress and strain curves (for wet state), in Figure 9, illustrate that the compressive strength improved with the addition of SSBC content into the composite polymer hydrogel-structures. PCMC/SSBC-0 hydrogel showed 0.46 MPa, whereas the incorporation of 10 wt % SSBC improved the compressive strength to 0.69 MPa. Higher SSBC content (30 wt% SSBC) further improved the compressive strength of PCMC/SSBC-30 hydrogels as 0.86 MPa. But for PCMC/SSBC-20 a little complex behavior was observed as compressive strength decreases (i.e., 0.64 MPa) as compared to PCMC/SSBC-10 and PCMC/SSBC-30, and this is possibly due to the agglomeration of ex-situ incorporation of SSBC particles which might have some brittle behavior under high compression due to porous nature of hydrogel even after incorporation of bioceramics. Jayarmadu et al. [45] have reported that the increasing water imbibing capacity leads to the softening of hydrogels which decreases the compressive modulus. The crosslinking between PAAm and CMC was improved with the addition of SSBC which enhances the mechanical performance of the hydrogel-network.

Therefore, for in vivo analysis, the high compressive performance of the SSBC incorporated composite hydrogels can facilitate improved load-bearing efficiency and can be considered as a suitable implant material for tissue engineering applications.

### 3.6. Zeta potential

The Zeta potential of synthesized composite hydrogels was evaluated by soaking them in SBF for 14 days. All the different compositions of hydrogels showed negative Zeta potential as shown in Table 4. The negative charge generated at the surface of the hydrogel scaffold is due to the carboxylate (COO$^-$) ions which attract the positively charged Ca$^{2+}$ ions released during the dissolution of ions with the formation of negatively charged silanol groups (SiOH) on the surface of SSBC on exposure with SBF (COO$^-$ Ca$^{2+}$), whereas PO$_4^{3-}$ ions from the surrounding medium of SBF get deposited on these positively charged Ca$^{2+}$ ions associated with the carboxylate groups (COO$^-$ Ca$^{2+}$ PO$_4^{3-}$) acts as a nucleation site for apatite formation [46]. Many authors inferred that the MC3T3-Eoseoblast cell adhesion and easy cell migration significantly depends on the negatively charged surface than the positively charged surface [47]. Thus, implant surface acquiring the negative Zeta potential provides a suitable environment for the osteoconduction in vivo.

### 3.7. In vitro antiproliferative activity

Selenium is a good anticancer agent which inhibits the growth of MG63 cancer cells in the body. The cell viability decreases with the increase in SSBC

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**Table 4. Zeta potential values of synthesized samples.**

| Sample         | Zeta Potential (mV) |
|----------------|---------------------|
| PCMC/SSBC-0    | −12.24              |
| PCMC/SSBC-10   | −14.76              |
| PCMC/SSBC-20   | −15.78              |
| PCMC/SSBC-30   | −16.24              |

**Figure 9.** Compressive stress–strain curves.
concentration which is due to the presence of selenium which has an inhibitory effect on the MG63 cancer cell line. PCMC/SSBC-0 hydrogel (without SSBC) shows the cell viability up to 89.7%, whereas PCMC/SSBC-10, PCMC/SSBC-20, PCMC/SSBC-30 hydrogels showed cell viability up to 74.4%, 60.3%, and 50.6%, respectively. Also, the proliferation curve of MG63 has been studied at different periods, such as 24, 36, 48, and 72 h for all the samples. This proliferation curve in Figure 10b depicts the inhibitory effect of selenium on MG63 cancer cell lines. The results were in good correlation with our earlier reported [21] and other studies. For example, Hu.et.al [48] have synthesized the selenium doped mesoporous bioactive glass (Se/MBG) nanospheres by a facile sol–gel technique and reported that Se/MBG nanospheres have an antiproliferative effect on the viability of MG63 osteosarcoma cells. These results indicate that

**Figure 10.** A) Cell viability graph (MTT assay) of hydrogel scaffolds cultured with MG63 cell b) MG63 cell line proliferation curves for different time intervals. Results are expressed as the mean ± SD (\(^{a}P \leq 0.05\) vs PCMC/SSBC-0; \(^{b}P \leq 0.05\) vs PCMC/SSBC-10; \(^{c}P \leq 0.05\) vs PCMC/SSBC-20.

**Figure 11.** Typical contour diagram of FITC-Annexin V/PI flow cytometry of MG63 cells treated with A:PCMC/SSBC-0, B: PCMC/SSBC-10, C: PCMC/SSBC-20, D: PCMC/SSBC-30 [necrotic (UL quadrant), live cells (LL quadrant), early apoptotic (LR quadrant) and late apoptotic (UR quadrant)].
the doping of hydrogels with the increased concentration of bioceramics containing selenium induces the death of cells of MG63 cancerous cell line.

Further, an Annexin V-FTIC/PI double staining assay was conducted to determine the qualitative assessment of cell apoptosis as provided in Figure 11. Different stages in the figure depicting apoptotic and necrotic MG63 cells were divided into four quadrants. Necrotic cells are shown in the upper part of the left quadrant (UL), late apoptotic cells in the upper right quadrant (UR), early apoptotic cells in the lower right quadrant (LR) and normal cells in the lower left quadrant (LL). The percentage of viable cells decreases with increasing concentration of bioceramics, i.e., PCMC/SSBC-10 ~75.1%, PCMC/SSBC-20 ~60% and PCMC/SSBC-30 ~47.3% whereas PCMC/SSBC-0 hydrogel has the maximum cell viability up to 87.3%. Therefore, it can be inferred that increasing the content of strontium and selenium doped bioceramics in hydrogels will enhance their anti-proliferative effect on the osteosarcoma MG63 cell line. Earlier studies even proved that the presence of selenium in bioceramics exhibits anti-cancer activity.

3.8. In vitro osteoblastic MC3T3-E1

The cellular proliferation of the cultured MC3T3-E1 osteoblast cells over the surface of pure polymer hydrogels and strontium and selenium doped bioceramics incorporated hydrogels incubated for 1 and 3 days is depicted in Figure 12. All samples exhibited the cellular viability of up to 76% but PCMC/SSBC-20 revealed that the cellular viability increases during 3 days as compared to 1 day (86.4% viable cells and 88.2% viable cells on 1 and 3 days) which indicates that the sample provides a favorable environment to the osteoblast cells for the maximum proliferation as compared to the other samples. These results indicate that the increase in the bioceramics content up to 20 wt% in the polymeric hydrogels enhances the cellular proliferation whereas increasing further content of bioceramics, i.e., up to 30 wt% lead to the decrease in cellular viability which is due to the increased selenium content in bioceramics provide the toxic environment to MC3T3-E1 osteoblast cells and lead to the cellular death [21]. This phenomenon was evident in the case of PCMC/SSBC-30 that indicated the cell viability 76.3% and 76.1% on 1 and 3 days in comparison to other samples. Although PCMC/SSBC-30 showed maximum anticancerous behavior, this sample is cytotoxic to normal MC3T3-E1 osteoblast cells whereas PCMC/SSBC-20 also showed good anticancerous properties and maximum osteoblast proliferation.

3.8.1. Osteoblast adhesion analysis by FESEM and cytoskeleton studies

The MC3T3-E1 cells exhibited the round shape morphology after day 1 incubation as depicted in Figure 14 (a-d). Many other authors speculated that MC3T3-E1 exhibited round shape morphology [49] and our reported study confirms it.

In addition to this, the cytoskeleton images of the cellular proliferation on pure hydrogel and bioceramics incorporated hydrogels for 1 and 3 days were taken (see Figure 15). The images indicate that PCMC/SSBC-20 exhibited uniform distribution and maximum cellular spreading which remain similar during 1st and 3rd days of cell culture as compared to the other samples which were due to the following reasons 1) Porosity and porous nature of hydrogel which induces the strong fixation of the MC3T3-E1 cells on a porous surface (see Figure 13c and 13g) with the easy cell

Figure 12. MTS assay demonstrating the cell viability and proliferation of MC3T3-E1 cells cultured on PCMC/SSBC-0, PCMC/SSBC-10, PCMC/SSBC-20, and PCMC/SSBC-30 hybrid hydrogels subsequent to 1 day and 3 days of incubation. Each value represents the mean ± SD (n = 3). Results are expressed as the mean ± SD. For day 1 (**P ≤ 0.05 vs control; *P ≤ 0.05 vs PCMC/SSBC-0; *P ≤ 0.05 vs PCMC/SSBC-10; *P ≤ 0.05 vs PCMC/SSBC-20; *P ≤ 0.05 vs PCMC/SSBC-30. For day 3 (**P ≤ 0.05 vs control; *P ≤ 0.05 vs PCMC/SSBC-0; *P ≤ 0.05 vs PCMC/SSBC-10.}
migration and vascularization, further leading to the proliferation and differentiation of MC3T3-E1 osteoblast cells for the growth of new tissue 2) Surface roughness of hydrogel which facilitate more osteoblast adhesion as compared to the smoother surface of the hydrogel. With the increase in bioceramics incorporation in polymeric matrix there is an increase in surface roughness which further results in osteoblast adhesion 3) Rapid apatite layer formation due to faster dissolution of ions from bioceramics on contact with SBF which facilitates osteoblast adhesion, proliferation, and differentiation on the surface of hydrogels [50,51]. PCMC/SSBC-30 with higher bioceramics content enhances the surface roughness but does not support the increased affinity for cellular adhesion due to the decreased porosity (Table 3). The incorporation of bioceramics up to 30% into polymeric hydrogel reduced the porosity as well as affects the porous nature and which leads to a decrease in the dissolution of ions and results in the reduced intensity of apatite formation as depicted by XRD diffractogram (Figure 2d).

3.8.2. Alkaline phosphatase (ALP) activity
ALP activity acts as an early marker for osteoblast differentiation in vitro which provides good assistance to determine the influence of osteoblast MC3T3-E1 cell adhesion on the surface of hydrogel to form new bone in vivo [52]. Figure 16 presents that osteoblast cells showed a positive dose-dependent expression of ALP on increasing the content of bioceramics in the samples as compared to pure polymer hydrogel at 7,10 and 14 days. Cells grown on PCMC/SSBC-20 sample showed significantly higher ALP activity on Day 10 (p = 0.042) and 14 (p = 0.021) as compared to PCMC/SSBC-0 and other samples. PCMC/SSBC-0 (pure polymer hydrogel) sample was not able to enhance the early osteogenesis indicator (ALP activity). Also, PCMC/SSBC-30 exhibited the increased ALP activity for the osteoblasts cultured on its surface for 7 and 10 days as

Figure 13. FESEM images of MC3T3-E1 osteoblast cell adhesion of MC3T3-E1 on pure hydrogel and bioceramics incorporated hydrogels for 1 and 3 days. a),e)PCMC/BMSS-0; b),f)PCMC/BMSS-10;c),g) PCMC/BMSS-20 and d), h) PCMC/BMSS-30.

Figure 14. FESEM images MC3T3-E1 osteoblast cell morphology on surface pure hydrogel and bioceramics incorporated hydrogels a) PCMC/BMSS-0 b) PCMC/BMSS-10 c) PCMC/BMSS-20 d) PCMC/BMSS-30.
compared to PCMC/SSBC-0 and PCMC/SSBC-10 but after 14 days, the ALP activity of PCMC/SSBC-30 is reduced as compared to PCMC/SSBC-10. These results were also supported by cell viability studies (see Figure 12) as well as cytoskeleton image (see Figure 15) where osteoblast proliferation and spreading decreased for PCMC/SSBC-30 after 3 days of culture which indicate the antiproliferative nature and reduced osteoblast differentiation (ALP activity) of sample increased with increasing culture time of osteoblast MC3T3-E1 cells on its surface. Therefore, PCMC/SSBC-20 exhibited maximum osteogenic potential which increased with the culture time and therefore, it may act as a suitable implant material for bone tissue engineering applications.

Keenan.et.al [53] have reported the following study. A bioactive glass series \(0.42\text{SiO}_2\cdot0.10\text{Na}_2\text{O}\cdot0.08\text{CaO}\cdot(0.40-x)\text{ZnO}\cdot x\text{Ga}_2\text{O}_3\) incorporated into carboxymethyl cellulose (CMC)/dextran (Dex) hydrogels in three different amounts (0.05, 0.10, and 0.25 m\(^2\)) was prepared. It was observed that the samples with increased Ga-content significantly decreased MG-63 osteosarcoma viability after 30 days. But our samples CMC/PAAm(PCMC) incorporated with strontium and selenium doped bioceramics (SSBC) i.e., PCMC/SSBC-10, PCMC/SSBC-20 and PCMC/SSBC-30 have shown

\[\text{Figure 15. Cytoskeleton images of osteoblast adhesion on surface of on pure hydrogel and bioceramics incorporated hydrogels for 1 and 3 days.}\]

\[\text{Figure 16. Alkaline phosphatase(ALP) activity of pure hydrogel and bioceramics incorporated hydrogels for 7,10 and 14 days. Each value represents the mean ± SD (n = 3). } ^a P ≤ 0.05 \text{ vs PCMC/SSBC-0 for that time point.}\]

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maximum inhibitory effect on MG63 cell line with in 72 h (see Figure 10b).

Suneetha. et al. [26] have synthesized hydrogels by incorporation of 0.25 and 0.5 g chitosan and sodium alginate-based polyelectrolyte complexes and reported about maximum compressive strength up to 0.45 MPa. But our sample with carboxymethyl cellulose and polyacrylamide, i.e., PCMC/SSBC-0 without any bioceramics has shown compressive strength up to 0.46 MPa. The higher mechanical strength is the major requirement for the clinical application of hydrogels for bone tissue engineering. Better compressive strength indicates the ability of the hydrogel to withstand the mechanical load without any deformation. This observation shows the ability of our synthesized hydrogels to provide better mechanical strength to the new soft tissues regenerated at the defect site.

4. Conclusions

In this study, the authors reported about the development of new bioceramics incorporated hydrogels for bone tissue engineering as well as for the recovery of bone cancer patients. The comparison analysis has been undertaken between the pure carboxymethylcellulose and acrylamide hydrogels and strontium and selenium doped bioceramics incorporated hydrogels. Samples with incorporated bioceramics have shown excellent bioactive behavior with significant apatite growth on the 1st day which can be considered as the positive factor for bone tissue regeneration and bone recovery from defects. The compressive strength of hydrogel scaffolds increased with the increasing doping amount of strontium and selenium bioceramics. However, the swelling rate and degradation rate decreased with the increased amount of doping of strontium and selenium bioceramics. PCMC/SSBC-20 sample’s composition can be considered as the best composition from this study due to the following reasons.

- PCMC/SSBC-20 has the optimum rate of degradation and swelling rate which is beneficial for bone recovery from defects and for clinical application of the hydrogels.
- This composition reports good apatite forming ability which induces osteoblast adhesion, proliferation, and differentiation for new tissue regeneration.
- The negative surface charge of hydrogel indicates the appreciable rate of apatite formation.
- PCMC/SSBC-20 has shown an anti-proliferative effect on cancerous osteoblast proliferation of the MG63 cancer cell line and can act as the anticancerous hydrogel due to the presence of selenium in the bioceramics and it can be used as a successful implant material for bone cancer patients for their recovery.

- PCMC/SSBC-20 provides a favorable environment for MC3T3-E1 osteoblast cell adhesion and proliferation. The cellular viability and proliferation were evaluated from quantitative analysis, i.e., cell viability test using MTS assay. The cell adhesion and spreading were analyzed by the qualitative analysis, i.e., cytoskeleton images and FESEM micrographs. Also, PCMC/SSBC-20 exhibited maximum ALP activity which acts as an early marker to evaluate osteoblast MC3T3-E1 cell differentiation for new bone formation. Therefore, PCMC/SSBC-20 has shown the highest osteogenic potential, and therefore, it can be further assessed for pre-clinical models for the treatment of bone defects. In future, authors may undertake in vivo studies using rats by using the chemical composition of PCMC/SSBC-20 sample.
- Authors have measured the apatite-formation ability in vitro and speculated that prepared hydrogel scaffolds have bone regeneration ability when implanted at bone defect sites. Additionally, ALP activity exhibited by the samples shows good osteogenic properties. Moreover, synthesized hydrogel scaffolds provide a conducive environment for normal osteoblast MC3T3-E1 cell proliferation. Therefore, based upon our preliminary findings, we have concluded that synthesized hydrogel scaffolds are speculated to be potential candidates for bone tissue regeneration applications. However, our results are focused on only in vitro assessment as preliminary results are needed for comprehensive in vivo analyses for the confident use of these hydrogel scaffolds for clinical testing. In vivo studies (by using rats) of our samples will be part of our future research curriculum to check the practical utility of our hydrogel scaffolds for bone regeneration applications.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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