Synthesis and Examination of Hydroxyapatite Nanocomposites Based on Alginate Extracted from *Azotobacter chroococcum* new strain MWGH-ShKB

**In vitro**

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Importance of bacterial alginate is attributed to its employment in multiple fields like pharmaceutical, biomedical and food industries. This research was aiming to discover a new Egyptian isolate of *Azotobacter chroococcum* and thereafter stimulation of production and extraction of alginate under various stress conditions. Extracted alginate then entered in a process of composition of hybrid inorganic composite based on an apatite and a polysaccharide of microbial origin. Isolates of *Azotobacter chroococcum* bacteria were isolated from different habitats in Egypt to assess the highest alginate productivity. Mannitol, sucrose, ethanol, tryptone, beef extract, peptone, yeast extract, pH, incubation time and agitation were tested for enhancement of alginate productivity. Biochemical tests and molecular study were done for the isolated *Azotobacter* and the new isolate was registered in GenBank with accession no MH179061. In vitro bone bioactivity was carried out via soaking the prepared materials in simulated body fluid (SBF) for 7 and 21 days in presence of hydroxyapatite nanoparticles (HA) and polymethyl methacrylate (PMMA) in different ratios. The examination of apatite formation on the prepared materials after soaking in SBF was carried out by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy with energy dispersive X-ray (SEM-EDX) and (3-(4,5-Dimethylthiazol)-2,5-Diphenyltetrazolium bromide) (MTT) assay was used to assess the cytotoxic effect of sodium alginate/hydroxyapatite analogs against normal splenocyte. The new strain was identified as *Azotobacter chroococcum* MH179061 which has the ability to maximize alginate production (3.8-4 g/L) in bacterial medium. Extracted sodium alginate has the ability of formation of a thick apatite layer on the surface of the composite films in special way on this containing SA/PMMA with ratio (1.5:1 and 1:1) compared to other ones. Significant increase in splenocyte proliferation noticed the nontoxic effects of the SA/HA particles, moreover, the cell division was stimulated; cells were able to maintain their healthy and being even in the presence of different concentration of HA particles more than control. The novel composites based on sodium alginate produced from *Azotobacter chroococcum* MH179061 has promising bioactivity properties, which can be applied in bone implants and tissue engineering applications after further investigations.

**Keywords:** Alginate, *Azotobacter*, nanocomposites, in vitro bone bioactivity, calcium/phosphate apatite

**INTRODUCTION**

The Gram-negative bacteria *Azotobacter* and *Pseudomonas* can synthetize the biopolymer alginate that has material properties appropriate for plenty of applications in industry as well as in medicine (Pacheco-Leyva et al., 2016). *Azotobacter* spp. is reported as safe bio-source strain according to its GRAS (Generally Regarded
As Safe) status and not only alginates production but also beta hydroxye butyrate (Haroun and Abdel-Hamid, 2015) and siderophore production (Dewidar et al., 2018) derived from this type of microorganism display contiguous sequences of L-guluronic acid residues similar to those of algal alginates (Wang et al., 2015). Alginic acid is an anionic linear polysaccharide and it’s composed of (I-4)-linked b-D-mannuronate (M-residues) and a-L-guluronate (G-residues) with different ratios (Lee et al., 2014). Moreover, alginates is characterized by low toxicity, high stability and it is an excellent biomaterial. So far, alginates based materials have been applied for not only food additives but also scaffolds and carriers of different biomolecules (Hay et al., 2009). Microbial polysaccharides have distinguished important biological functions, as intracellular storage, as envelope, or as extracellular polymers such as xanthan, cellulose, and chitosan, (Schmid et al., 2015). Alginates has lots of applications in different industries due to its gelling and viscous character. Furthermore, in wastewater treatment, alginates can remove various pollutants such as heavy metals, dyes, and phenolic compounds (Elkady and Hassan, 2015). Alginates acts as a diffusion barrier for nutrients and oxygen (Setubal et al., 2009). Apatite is one of a few minerals produced by biological micro-environmental systems. Hydroxyapatite (HA), is the major component of tooth enamel and bone mineral. Dorozhkina, (2015 a) reported that HA is one of calcium phosphates which possess the highest crystallinity and has been used in bone repair operation due to its high strength of binding with original bone tissue, high tolerance for enzymatic degradation, and its dissolution is pH-dependent. Uskokovic and Uskokovic (2011) clarified that HA also can stimulate the differentiation of osteoblasts migrating into the scaffold (Wang et al., 2015). Haroun et al., (2010, 2010, 2013), reported that the combined gelatin/HA and beta-cyclodextrin/HA nanoparticles based composites were prepared for osteogenesis and had higher osteoblast attachment and proliferation than HA, MTT assay as a colorimetric method that measures the reduction of yellow by mitochondrial succinate dehydrogenase and quantitative cytotoxicity assay for screening purposes, because the cellular reduction is only catalyzed by living cells, it is possible to quantify the percentage of living cells in a solution (Riss et al., 2011). The main target of this research is isolation and identification of new Egyptian isolate of Azotobacter chroococcum, and maximization of alginates production as a new source for medical applications which is formation of reticulated hybrid inorganic composite based on an appetite and a polysaccharide of microbial origin.

**MATERIALS AND METHODS**

Isolation and Identification of the alginate producing bacterium

Fifty grams of soil sample from Shoubra El Kheima, (30°7′24.19″N and 31°15′39.34″E) Qalubia Governorate, Egypt was used to isolate Azotobacter sp. which has been made on the basis of its alginate production (Gauri et al., 2009). Liquid Atlas medium (Atlas, 1997) was used to enrichment of Alginic acid producing bacterium (Azotobacter sp). Glucose 20; K2HPO4 0.8; MgSO4 0.5; KH2PO4 0.2; FeCl3 0.05; CaCl2 0.05; NaMoO4.2H2O 0.05; distilled water 1000ml and pH adjust 6.8-7. The bacterial genomic DNA was extracted from a fresh cell culture which was used for PCR of 16S rRNA genes followed by sequencing as molecular confirmatory test. Sequences of the 16S rRNA universal primers were used 16S rRNA (F) 27F (5′-GAG AGT TTG ATC CTG CAG-3′) and 16S rRNA (R) 1495R (5′- GAG CTA CTA CCA GCT ATT-3′) (Studholme et al., 1999) and Sanger 1977. The PCR conditions were performed at the following temperature cycles: 5 min initial denaturation at 95°C, followed by 30 cycles of denaturation at 94°C for 35 s, annealing at 57°C for 30 s, and extension at 72°C for 60 s, final extension at 72°C for 10 min. A total of 5 µL of PCR products were analyzed. A total of 5 µL of PCR products were analyzed by 1% agarose gel electrophoresis and made visible by ethidium bromide (0.5 mg/mL) staining and UV transillumination. The PCR amplified DNA was eluted from gel and purified by QIA quick gel extraction kit (QIAGEN). Sequencing was done using Bigdyte terminator kit (ABI) and same primers (used for PCR) in an automated DNA sequence (ABI model 3100, Hitachi). The sequence was then compared with those available in the GenBank databases using BLAST (Pearson and Lipman, 1988) and phylogenetic analysis was done using MEGA version 3.1 program by NJ (Neighborhood Joining(MP (Maximum Parsimony) method (Kumar et al., 2004)).

Maximization of alginate production and extraction of sodium alginate

Different Carbon sources were added individually to the Atlas medium except control to

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*Preparation of new nanocomposite of Azotobacter chroococcum alginate*
determine the effect of carbon dose on alginate production by the strain (glucose 2% as control, 1% mannitol; 2% lactose; 2% glucose and 1.6% ethanol) were introduced each in equimolecular carbon content as sole carbon source. Effect of different nitrogen sources tryptone 1%; meat extract 1% and peptone 1% on EPS production was checked. Studying the limit variation of pH the medium was adjusted to 4, 6.9, 8 and 9. Three agitation speeds (150, 200 and 300 rpm) were compared with static condition was examined. Incubation period: using incubator with temperature range 20-40°C was also estimated. Alginate production was determined according to Sabra et al., (1999) for each treatment; at least two samples were used. Biomass dry weight of the bacterial strain was measured according to (Galal and Ouda 2014). Standard inoculum containing 1x10⁷ cells was inoculated into ten conical flasks containing 100 ml Atlas medium and all flasks were incubated at 28 °C for 72 hrs. Cells were harvested from the cultures by centrifugation at 5000 rpm /20min. The cells were dried at 70°C for 48 hrs. Then, cells were weighted and calculated per 1 liter. Identification and detection of alginate were estimated by United States pharmacopeia Identification technique.

Preparation of nanocomposite based on extracted sodium alginate

Different concentrations of the produced sodium alginate (0, 10, 4.5 and 3.0 wt%) aqueous solution mixed with different concentrations of polymethyl methacrylate (0, 9, 3, 3 wt%) separately in presence of sodium dodecyl sulphate(SDS), ceric ammonium nitrate and hydroxyapatite nanoparticles (HA) under stirring to afford emulsion system under sonication treatment followed by addition of 1mL epichlorohydrin as cross-linker(Bakhtiari et al 2016; Dorozhkin 2015b,Turon et al., 2017;Habraken et al., 2016; Landi et al., 2007). The obtained emulsions were separated by centrifuge, dried and kept for further investigations (Table 1).Preparation of SBF was carried out where In 1L beaker containing 750ml ultra-pure water reagents were added one by one as the order of Table 2 then stirred, pH adjusted at 7.4 and stored at 5-10 °C for further investigations (Lee et al., 2011 and Xiaobo et al., 2008).

Partial characterization of the prepared materials

Fourier transform infrared spectrometry FTIR was recorded different functional groups of samples using 4100Jasco-FTIR. Scanning electron microscope (SEM)/ Electron Dispersive Spectroscopy(EDX) were used to determined morphology and structure of the prepared nanocomposite using Quanta FEG250-SEM.

Table (1) Chemical composition of the prepared materials

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Chemical composition (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sodium alginate (SA)</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>4.5</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Sample code</th>
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</tr>
<tr>
<td>B</td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Table (2) Simulated body fluid (SBF) buffer components

<table>
<thead>
<tr>
<th>Material</th>
<th>Qty. g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
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</tr>
<tr>
<td>NaHCO₃</td>
<td>0.35</td>
</tr>
<tr>
<td>KCl</td>
<td>0.224</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>0.228</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.305</td>
</tr>
<tr>
<td>HCl (1kmol/m³)</td>
<td>40cm³</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.278</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>0.071</td>
</tr>
<tr>
<td>(CH₂OH)₃CNH₂</td>
<td>6.057</td>
</tr>
<tr>
<td>HCl (1kmol/m³)</td>
<td>Appropriate amount</td>
</tr>
</tbody>
</table>

Splenocyte proliferative activity

Three months-old male CD1 mice (completely normal free of any pathogen) were purchased from faculty of veterinary medicine, University of Sadat City, Egypt. Normal mice were sacrificed and their spleens were removed under completely aseptic conditions (Mosmann 1983). Single cell suspensions were prepared by forcing splenic tissue through stainless steel mesh and red blood cells were lysed using Ammonium-Chloride-Potassium (ACK) lysis buffer (Sigma Chemical Co., St. Louis, MO, USA). The cells were then washed twice (by centrifugation for 10 min at 2000 X g) and re-suspended in incomplete RPMI-1640 medium (RPMI-1640 medium was supplemented with L-glutamin (200 mM), penicillin (100 U/ml), streptomycin (100 lg/ml) and HEPES buffer (1 M). The viability of the cells was measured using
trypan blue stain (Sigma) and was ~90%. The cells were adjusted at a concentration of 4×10^5 cells/well in complete phenol-red free RPMI-1640 medium supplemented with 10% heat inactivated mycoplasma- and virus- free fetal bovine serum (FBS). The cells were cultured in triplicates in a volume of 100 μl/well into flat- bottom 96- well tissue culture plates. Splenocytes were incubated with various concentrations of the produced alginate and nanocomposite sample (0.5 and 0.25 mg/ml) for 96 h successive days at 37°C in humidified air containing 5% CO₂. The effect of hydroxyapatite nanocomposite on splenocytes proliferation was estimated by MTT assay according to Talaat et al. (2015). Forty μl/well of MTT (5 mg/ml in 0.9% NaCl) were added to the culture and incubated at 37°C for additional 4 h. Metabolically active cells form insoluble purple formazan crystals were solubilized by adding 180 μl/well of acidified isopropanol (0.04 N HCl in absolute isopropanol) and incubated for 24 h at 37°C. The absorbance at 570 nm was measured. The results are expressed as the mean percentage of viable cells as compared to the respective control cultures treated with the antigen-free media. The half maximal growth inhibitory concentration (IC₅₀ values) was calculated from the line equation of the dose-dependent curve of each compound. All culture materials were obtained from Biowest (Rue du Vieux Bourg, Nuaillé - France).

**Statistical analysis**

All statistical analyses were performed using SPSS version 13 (SPSS, Inc., Chicago, IL). Comparisons between different groups were performed by T-test. In all tests the level of significance was set at p<0.05.

**RESULTS AND DISCUSSION**

Identification and phylogenetic analysis of *Azotobacter chroococcum*

The biochemical tests revealed that the isolate is aerobic, catalase, starch and urea hydrolysis positive and utilize different carbon sources such as glucose, mannitol, inositol, rhamnose, arabinose, ethanol, sorbitol, butanol and gluturate, so it could be regarded as *Azotobacter* sp. The isolates were classified as *Azotobacter chroococcum* according to the above criteria of Bergey’s Manual of Systematic Bacteriology (Brenner et al., 2005) (Fig. 1).

The partial sequence of 16S rRNA gene sequence comprises of approximately 1.5 kbp has been so far reported (Gimmestad, et al., 2006). A (~1.5 Kbp) Amplified fragment was sequenced and total 1278 kbp (nucleotide) sequence was determined and register in GenBank with accession number as MH179061 under title *Azotobacter chroococcum* strain MWGH-ShKB. Phylogenetic analysis of this obtained sequence showed close relationship of MWGH-ShKB with KF494187.1 *Azotobacter chroococcum* ABA-1 (Fig. 2).
carbon sources and it is important for cyst formation (Sabra and Zeng 2009; Flores et al., 2013).

In addition, there was significant higher alginate production rate along with the addition of peptone (4g/L) in comparison with control (without nitrogen source) and utilizing tryptone, beef extract, and yeast extract which yielded 1.8, 1.2 and 3.4g/L respectively. Addition of peptone to Atlas medium enhanced production of alginate and biomass dry weight (4 and 12 g/L) in comparison with nitrogen source free medium (1.8 and 5.4g/L) and provides better available nitrogen for the bacterial growth and alginate production. This result supported by the results recorded by Khanafari and Sepahei (2007). Also, Anyanee 2008 noticed that addition of inorganic nitrogen led to enhance growth of Azotobacter sp. Moreover, significant increasing along with yeast extract and ammonium nitrate as organic and inorganic nitrogen sources showed highest production Pandurangan et al., 2012.  

The variation in growth rate related to the pH presents an optimum value and extreme limits. Controlling pH value at 7.2 during cell cultivation showed positive effect on cell productivity (1.8 g/l). Furthermore variable pH values were examined to increase alginate yield where, pH 8 was found to produce 2.1g/L alginate with 6.4 g/l biomass in comparison with acidic pH (0.1g/L) it may due to drastic pH inhibition effect. Earlier Gauri et al., 2009 clarified that Azotobacter sp. prefers to grow at alkaline pH 7.5–8.0. Hydrogen ion concentration has a significant influence on microbial fermentation due to its importance in controlling bacterial growth, transfer the nutrients through the cell wall and product formation. This pronounced result may be due to the heterogeneous pO2 profile during the fermentation run in flasks as mentioned by Sabra et al. 1999.

It was observed that agitation improved the dissolved oxygen in the culture medium which reflected on Azotobacter sp. growth and alginate production. Alginate production 3.6 g/L and 11 g/l biomass was achieved with 300rpm agitation speed. As shown in Fig. (3) it was noticed that the quantity of sodium alginate was slightly affected by lowering agitation speed to 150 producing 2.4 and 3.5g/L respectively. While in static condition there was insufficient oxygen in the culture medium which reduced the amount 1.8g/L in compare to agitation condition as mentioned in Abdel-Hamid et al., 2012. Directly proportional with incubation time was noticed that during early times of incubation 2 days, bacterial biomass was 3.2g/L and sodium alginate was 1g/L. Furthermore, alginate concentration was increased until 7 days, at which sodium alginate was 3.1g/L along with 11.2 g/l biomass. Accordingly, alginate yield based on carbon source utilization was increased to became 2.2, 2.7 and 3 g/L after 4, 5 and 6 days respectively Fig. (3).
Figure.(3) Effect of different stress conditions on sodium alginate production from *Azotobacter chroococcum* new strain MWGH-ShKB
Partial characterization of the prepared nanocomposites based on alginate

The bone-bonding ability of the composite films was evaluated by examining the ability of apatite to form on their surface in SBF with ion concentrations nearly equal to those of human blood plasma. Results of FTIR in showed that Fig (4) the characteristic peaks of (PO₄)³⁻ and (–OH) groups which corresponding to hydroxyapatite are existed in the prepared composites after soaking in SBF for 21 days. SEM-micrographs show photographs of SA/HA based nanocomposite surfaces (Figs 5 and 6). It can be concluded that typical features of precipitation of Ca/P after soaking in SBF for 7 and 21 days. Precipitation started at individual granules on the PMMA/HA coated SA substrate and the granules gradually grew together to form a dense hypothesized that soaking in SBF solution provides a supersaturation of Ca²⁺ ions around the coated SA substrate through ionic interactions between calcium ions and the negatively charged –OH groups available on the SA. Then the incorporated phosphate ions bind to calcium ions to form the initial nuclei. Once the Ca/P nuclei are formed, they grew by uptake of calcium and phosphate ions from the surrounding SBF Ge et al., 2010. The spherical and needle-like particles of SA/HA based nanocomposites are observed in SEM images Figs (5 and 6). Palin, et al 2016 revealed that bacterial based beads can swell and form a network, with a calcite–alginate bio composite which, can stimulate great possibility for the development of self-healing concrete in low-temperature marine environments. In other words, soaking in vitro in SBF demonstrated that the prepared composite materials had reasonably good bioactivity. Generally, SA/HA-based nanocomposites are suitable for use in bone defects or regeneration of bone. Fig (7) and Table (3) shows EDX elemental analysis of the prepared materials. It can be seen that Ca/P concentration ratio at different sites of the prepared materials using energy-dispersive X-ray spectroscopy.

Figure (4). FTIR spectra of the prepared materials (a) before and (b) after soaking in SBF for 21 days where Blank (PMMA:HA), 1(SA), 2 (SA:PMMA) and 3(SA:PMMA:HA)
Figure (5) SEM-micrographs of the prepared materials before and after soaking in SBF for 7 days (X30µm, 20 kv) where Blank (PMMA:HA), 1(SA), 2 (SA:PMMA) and 3(SA:PMMA:HA)
Figure(6) SEM-micrographs of the prepared materials before and after soaking in SBF for 21 days (X30µm, 20 kv) where, Blank (PMMA:HA), 1(SA), 2 (SA:PMMA) and 3(SA:PMMA:HA)
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Fig (7) EDX analysis of the prepared materials after soaking in SBF for different time intervals where Blank (PMMA:HA), 1(SA), 2 (SA:PMA) and 3(SA:PMA:HA)
Table (3) EDX analysis of the prepared materials after soaking in SBF for different time interval

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Elemental analysis (wt%)</th>
<th>After 7 days</th>
<th>After 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>P</td>
<td>Ca</td>
</tr>
<tr>
<td>B</td>
<td>1.88</td>
<td>0.96</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>1.55</td>
<td>5.7</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>7.3</td>
<td>4.1</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>6.7</td>
<td>3.7</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Blank (PMMA:HA), 1(SA), 2 (SA:PMMA) and 3(SA:PMMA:HA)

The relative content of Ca and P is critical for sustaining mineral homeostasis and bone metabolism and their co-dependence is evident for bone growth and development Haroun et al., 2010. Alginic acid chelated the different metals and formed a reversible gel-like structure named egg-box Li et al., (2007). The alginate based metronidazole periodontal gel has promising release properties as antibacterial activity, high stability at room temperature for 3 months. Sinurat et al., 2014 addition of nano fibrillated cellulose (NFC) increased a tensile strength of the film from 20%. Sirviö et al., 2014 reported that fabricated of microspheres from biopolymers possess several advantages for use in biomedical applications which can improve delivery of the spheres to the specific target site, along with larger surface area allowing for sufficient therapeutic coatings; an increase in degradation rate; ion release and allowing encapsulation of other biomedical relevant components.

Cytotoxicity and MTT assay

In order to confirm the nontoxic effects of our sodium alginate or biopolymer SA/HA MTT assay as a direct contact assay and live/dead assay using ethidium bromide/acrydine orange (EtBr/AO) cock tail were done. The spleen cells grown in the presence of even a concentration of both sodium alginate and SA/HA nanocomposite (0.25 mg/ml and 0.5 mg/ml) the concentration 0.5 mg/ml exhibited an increase in viable cell count 141.03% more than concentration 0.25 mg/ml which produced 87.42% in comparison with viable cell count reach to 101.43% and 99.3% with (0.5 and 0.25 mg/ml respectively Table 4 and (Fig 8).

Table (4) In vitro proliferative activity of sodium alginate on splenocyte cells using MTT assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.5 mg/ml</th>
<th>0.25 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracted SA</td>
<td>101.43</td>
<td>87.42</td>
</tr>
<tr>
<td>SA:PMMA:HA</td>
<td>141.03</td>
<td>99.3</td>
</tr>
</tbody>
</table>

* Numbers referred to % of viable cell count.

These results confirmed the nontoxic effects of the SA/HA particles moreover, confirmed that the cell division was stimulated; cells were able to maintain their healthy and being even in the presence of a different concentration of HA particles. HA was already proven to be highly compatible and this bioactive inorganic material has been used for several biomedical applications Smitha and Usha 2016.

Figure (8). In vitro proliferative activity of sodium alginate on splenocyte cells using MTT assay
The compatibility of HA is due to the similarities in chemical composition, size, crystallinity and morphology to bone minerals and the non-toxicity of HA (Muzzarelli 2011). In vitro anti-inflammatory activity of sodium alginate and hydroxyapatite on splenocyte cells (Splenocytes proliferation) using MTT assay was summarized in Fig 8 and Table 4. Examination the viable cell count of the fabricated SA/HP microspheres exhibited excellent biocompatibility, because even when the dosage can be observed that the maximum cell growth was recorded at ratio (1.5:1.0) of SA:PMMA in presence of 1.0 wt% HA. On the contrary, maximum reduction was observed at 0.25 mg/ml of sodium alginate as Wang et al., 2015. Cytotoxicity tests indicated that the prepared composite could promote not only cell proliferation and stimulate cell division but also biocompatible; the same results were previously recorded by Lia et al., 2018. Splenocytes proliferation and adherent monocytes (MQ) mediators were always used to screen the potential immune modulatory effect on a substance Seo et al., 2005. Splenocytes consist of a variety of immune cell populations including T and B lymphocytes, dendritic cells, and MQ, which have different immune functions. In the presented results, SA/HA-based nanocomposites gave maximum stimulation of splenocytes proliferation. The MTT can be used to evaluate the cytotoxicity of: Extractable materials of medical devices, Toxic compounds, Toxins and environmental pollutants. Potential anticancer drugs and Antibodies to examine growth inhibiting potential Riss et al., 2011.

CONCLUSION

This study focused on isolation and identification a new strain of Azotobacter chroococcum with accession no MH179061 that could maximize alginate yield. Employment extracted alginate with the synthesized hydroxyapatite nanocomposite to construct osteogenesis. Furthermore, not only successful fabrication of SA/HA nanocomposites particles with optimized reaction conditions is a great challenge but also the cell viability test evidenced that the fabricated SA/HA nanocomposites had promising new source biocompatibility.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest'.
Gimmestad M, Steigedal MH et al. 2006 Identification and characterization of an Azotobacter vinelandii type I secretion system responsible for export of the algE-type mannanuronan C-5-epimerases. J Bacteriol. 188:5551–60


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