Osteoconductive properties of synthetic eggshell hydroxyapatite: an experimental study in rats

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Abstract

Purpose – This study examines the osteoconductive and healing capabilities of locally implanted synthetic hydroxyapatite (sHAp) derived from eggshells in the central incisor sockets of rats.

Design/methodology/approach – Toxicity experiments were conducted in vitro and in vivo, to testify the safety dosage of sHAp. Around 24 mature male Sprague–Dawley (SD) rats had their upper central incisors extracted. The rats were placed into three groups of eight rats each: Group 1: the sockets of extracted central incisors were left unfilled (control), Group 2: filled up with commercially available hydroxyapatite (HAp) and Group 3: implanted with sHAp locally retrieved from eggshells. After extraction, four rats from each group were sacrificed at 2nd and 4th weeks. Maxillary tissue sections were obtained and stained with hematoxylin and eosin (H&E) and Masson's trichome (MT) staining. Anti-osteocalcin (OCN) and proliferating cell nuclear antigen (PCNA) were used primary antibodies for immunohistochemistry (IHC) special labeling.

Findings – The results showed that the locally implanted sHAp was non-toxic and safe in cell lines (human osteoblast and fibroblast) and animals. Histological analysis of H&E, MT and IHC showed that the sockets treated with locally implanted sHAp from eggshells were filled with new bone tissue of comparable thickness to other groups.

Originality/value – This unique technique uses locally implanted eggshell-derived sHAp with osteoconductive characteristics. In an in vivo model, sHAps increased OCN and PCNA expression to improve bone repair.

Keywords Osteoconductive, Eggshell, OCN, PCNA

Paper type Research paper

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1. Introduction

Patients, as well as donors, are traditionally the main sources for replacing missing bones (Damien & Parsons, 1991). However, several factors including the death of a donor, the skills required for second surgery harvest graft, the insufficient availability for autograft and the fact that the procedure is time-consuming (Clavero & Lundgren, 2003; Greenwald *et al.*, 2001), may deteriorate the healing process of a replaced bone. Traditional grafting methods have been gradually eliminated over the years and replaced by the usage of synthetic or processed bone graft substitutes (Greenwald *et al.*, 2001; Hench & Wilson, 1993). Thus, clinicians and scientists in the field of dentistry have been thoroughly researching as well as developing biocompatible materials readily extracted from animals and their by-products to be used as viable bone graft substitutes.

Hydroxyapatite (HAp), $Ca_{10}(PO_4)_6(OH)_2$, is one of the most stable forms of calcium phosphate found in the inorganic component of enamel and human bone (Greenwald *et al.*, 2001; Kattimani, Lingamaneni, Chakravarthi, Sampath Kumar, & Siddharthan, 2016). HAp has been widely utilized as an implant material for many years due to its excellent biocompatibility and bone-bonding ability and also due to its structural and compositional similarity to the mineral phase of hard tissue in human bones. The implantation of HAp into bone reveals that the material is safe and has excellent osteoconductive properties biologically, histologically and biomechanically (Yamamoto, Onga, Marui, & Mizuno, 2000). Besides that, according to Vallet-Regí (Vallet-Regí, 2001), HAp appears to be the most promising agent due to its outstanding biological properties such as lack of toxicity, lack of inflammatory response and absence of fibrous or immunological reactions. The bioactive nature of HAp gives rise to a favorable reaction product through a chemical transformation of its starting material to the desired final product. Subsequently, when HAp comes in contact with physiological fluids, a chemical reaction initiates the production of newly formed bone known as an osteoconductive process (Vallet-Regí, 2001). Additionally, the synthetic form of the inorganic bone substitute is shown to be chemically and crystallographically similar, although not identical to the natural hydroxyapatite. In the field of implant dentistry, strategies might be considered to preserve ridge involving modulation of the physiologic modeling process that occurs after tooth extraction (Allegrini *et al.*, 2014).

Chicken eggshell membrane and powder have the most similar and promising physical properties expected as an osteoconductive agent. Bone healing has been observed after being treated with eggshell powder (Kim *et al.*, 2008; Lee, Kim, Balázsi, Chae, & Lee, 2012; Park *et al.*, 2008), which further confirms eggshell powder as an osteoconductive bone filling material of significant value in bone regeneration. Several previous reports confirmed that properties of eggshell-derived HAp are ideal compared to commercially available graft materials (V. S. Kattimani *et al.*, 2014; Siva Rama Krishna, Siddharthan, Seshadri, & Sampath Kumar, 2007). It is suggested that eggshell exhibits favorable characteristics as a biomaterial for bone grafting interventions, owing to its biocompatibility and osteoconductive attributes (Opris *et al.*, 2020). A prior investigation conducted a comparative analysis of the efficacy of demineralized bone matrix in conjunction with guided tissue regeneration membrane versus eggshell constituents and its membrane as a means of promoting regeneration in Wistar rats (Kavarthapu & Malaiappan, 2019). The findings indicated that both substances exhibited efficacy in stimulating fresh osseous tissue generation and complete defect restoration while causing minimal epithelial entrapment. The research findings suggested that eggshell powder, in conjunction with its membrane, exhibits promise as a viable graft material. Furthermore, the biocompatibility of osteoblastic cells with HAp material derived from eggshells and subjected to silicon (Si) and poly (lactic-co-glycolic) acid (PLGA) modifications is significantly high (Gutierrez-Prieto *et al.*, 2019). The HAp/Si/PLGA composite exhibits potential as a viable candidate for the fabrication of 3D scaffolds intended for dental bone regeneration. The bioactive and biocompatible nature of the material renders it suitable for potential utilisation in the fabrication of three-dimensional scaffolds, which could serve as a crucial component in the process of bone regeneration. The substance exhibits properties of self-sustainability and affordability and holds potential for eventual use in clinical contexts within the domains of medicine and dentistry.

Former histological analysis suggested that the rate of rat alveolar healing is completed by the end of 3rd week after tooth extraction (Sadr, Aghbali, Abachizadeh, Azizi, & Mesgari Abbasi, 2017). Moreover, extracted tooth socket that is filled with material exhibited more bone trabeculae surrounding vascular structure to form primary osteon, by 21 days. However, quantitative analysis showed an increase in new bone formation up to 6th or 8th week with a major proportion of it taking place by the end of the 2nd week (Allegrini *et al.*, 2008). In addition, blood coagulum formed by breakage of a blood vessel from the periodontal ligament and apical foramen is noticed in the extracted socket shortly after tooth extraction.

Therefore, the present study is aimed to investigate the potential positive histological effects of locally implanted synthetic hydroxyapatite (sHAp) extracted from eggshells delivered as a novel bone substitute post extraction of central incisors of rats.

2. Methods

2.1 Preparation of the avian eggshell powder as a filling material

sHAp extracted from chicken eggshells were pulverized into nano-like particles by the Faculty of Mechanical Engineering of Universiti Teknologi Mara through advanced procedures (Bin Syed Mohd Hassan *et al.*, 2013; Shakir, Al-Bayaty, & Albajalan, 2019).

2.2 Ethics statement

This study was approved by the Research Committee on the Ethical Use of Animals, Universiti Teknologi Mara, Malaysia (NO 25/2013-600-FF (PT 5/2). All surgery processes were done under sterile conditions.

2.3 MTT assay on cell lines

Fibroblasts and osteoblast cell lines have been selected for this project. According to Claeys' (2020) research, fibroblasts exhibit a high degree of suitability for the generation of osteogenic cells within preclinical models of bone biology (Claeys, Bravenboer, Eekhoff, & Micha, 2020). In Addition, it was proposed to utilize osteoblasts in osteoconductive research due to their role in bone synthesis and mineralization during the early stages of bone formation and subsequent bone remodeling Rosenberg, Rosenberg, & Soudry, 2012; Pirraco (2012), found that fibroblasts regulate osteoblast behavior through gap junctional communication (Pirraco, Cerqueira, Reis, & Marques, 2012). Overall, the fibroblasts and osteoblasts interact and influence each other's activity, which may have implications for bone regeneration and tissue engineering approaches. The MTT assay can be used to evaluate the osteoconductive activity of biomaterials on osteoblast and fibroblast cell lines. Arbez (2017) found that the MTT assay was effective in measuring the proliferation of osteoblasts on a beta-tricalcium phosphate biomaterial (Arbez & Libouban, 2017).

Fibroblast cells BJ (ATCC1 CRL-2522TM) and osteoblast (406-05FHuman Osteoblasts: HOb, fetal), were cultured at 37 °C in 5% CO_2 atmosphere in Minimum Essential Medium Eagle Alpha (a-MEM). About 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin were the main ingredients of the medium. During the confluence cells, 0.02% EDTA–0.05% trypsin solution was used for sub-culturing the cells. The cells were cultured in the presence of agents and/or H_2O_2 for 48 h.

2.4 Cell viability

A 96-well cell culture plate was used. A day before the experiment, 5,000 cells per well were seeded approximately. About 100 ml of growth media were used for seeding the cells and treated with (0.8, 1.61, 3.12, 6.25, 12.5 25 and 50) ml of sHAp. The treatment was kept for 48 h, after which, a 10-ml MTT solution (5 mg/ml) was added to each well, and incubated for 4 h at

37 °C. Before the addition of 100 ml of dimethyl sulfoxide (DMSO), the spent media and MTT were aspirated for adherent cells, to dissolve the MTT formazan. Absorbance was read at 570 nm after 5–10 min of incubation.

$$\text{viability}\,\% = \frac{\text{OD sample}}{\text{OD blank}} \times 100$$

2.5 Acute toxicity

This experiment has been conducted to testify the safest dosage of sHAp. Around 36 Sprague–Dawley rats (18 males and 18 females) were organized into three groups and treated with vehicle (0.5% CMC, 5 ml/kg) or given 50 or 100 mg/kg of sHAp (5 ml/kg) orally. The rats were excluded from being fed a night before the administration of sHAp. The rats were monitored for 48 h to observe potential adverse symptoms (due to toxicity) exhibited by the rats. On the 15th day, xylazine and ketamine were applied for anesthetic purposes. Histological, hematological and serum biochemical parameters were assessed, following the standard methods (Al Batran *et al.*, 2013; Bayaty, Zaidi, Abdullah, Emad, & Al-Obaidi, 2018).

2.6 Experimental animals and surgical techniques

First, the rats were anesthetized with intramuscular injection (0.59 ml/kg) of a combination of ketamine hydrochloride and xylazine. Next, the upper central incisors of the 32 rats were extracted as previously described (Al-Obaidi, Al-Bayaty, Al Batran, Hassandarvish, & Rouhollahi, 2014). The rats were divided into three groups; four rats in each group.

Group 1-: The sockets of extracted central incisors were left empty where the sockets were allowed to heal on their own.

Group 2-: The sockets of extracted central incisors were filled with commercially available HAp (purchased from Sigma Aldrich, USA).

Group 3-: The sockets of extracted central incisor were filled with local sHAp obtained from the eggshell.

All of the extraction sockets were sutured using resorbable dental suture 5-0. Due to the small size of the sockets in rats, the loop was used to aid the vision of the operators. The instruments used during suturing were artery forceps, end-cutting needle, resorbable suture and scissors. Three rats from each group sacrificed at the 2nd and 4th week, post extraction of the central incisors.

2.7 Histological investigations

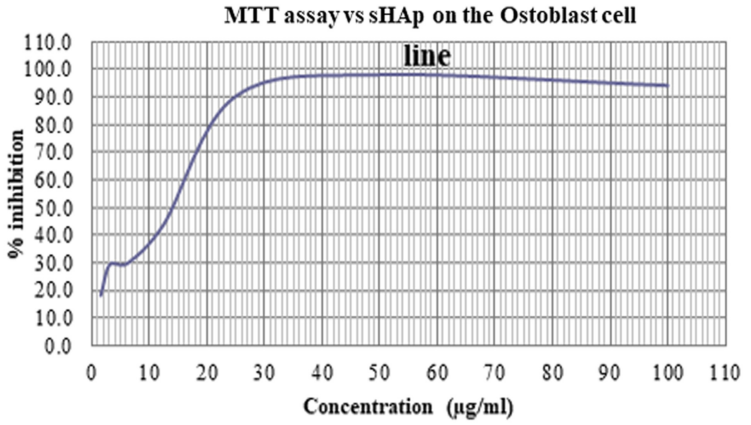
The maxilla of each deceased rat was sectioned and fixed in 4% neutral formalin for 48 h (Bayaty *et al.*, 2018). After that, the maxilla was demineralized in formic acid and sodium citrate for 5–7 days and embedded in paraffin wax. For performing the histopathologic examination, serial cross-sections at 5 μm in a longitudinal direction were stained with H&E (Sigma, Aldrich) and Masson's trichome (MT). Additionally, the immunohistochemistry (IHC) staining technique was also applied using primary antibody an anti-OCN (osteocalin) and anti-PCNA (proliferating cell nuclear antigen). At last, the results have been presented based on the qualitative and quantitative analysis (Al-Obaidi *et al.*, 2014) of data of the newly formed bone tissue. MT staining was done on week 2, while H&E and IHC were carried out on week 4.

2.8 Statistical analysis

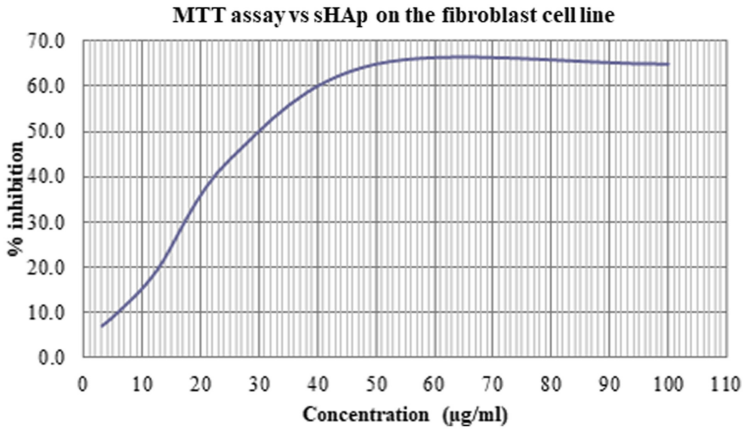
Independent-sample *t*-test was conducted to compare the effect of different treatments vs control group. SPSS 20 was used to perform the statistical analysis. Asterisk (*) indicates the significance level $P < 0.05$, when compared to control group.

3. Results

Experiments evaluating the toxicity of sHAp extracted from eggshells were conducted on human osteoblast and fibroblast cell lines. The results of these experiments, as depicted in Figure 1A and 1B, demonstrate that the sHAp obtained from eggshells exhibited no toxicity towards these cells. Also, the numbers show that the presence of eggshells increased the viability of osteoblast and fibroblast cells in vitro, causing these normal cells to multiply in a way that depends on the dose.



(a)



(b)

Note(s): (A) Viability of human osteoblast cells treated with sHAp extracted from eggshells. The data indicates that the sHAp showed no toxicity towards the osteoblast cells. The viability of the cells increased in a dose-dependent manner. (B) Viability of human fibroblast cells treated with sHAp extracted from eggshells. The results demonstrate that the sHAp exhibited no toxicity towards the fibroblast cells. Similar to the osteoblast cells, the presence of eggshells increased cell viability in a dose-dependent manner. CTRL = dH2O

Source(s): All the figures were made by authors

Figure 1. Evaluation of sHAp toxicity on human osteoblast and fibroblast cell lines

3.1 Acute toxicity analysis

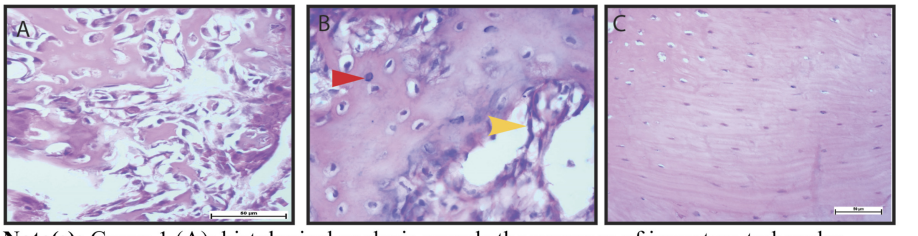
At the managed dosages, the animals did not show any signs of toxicity (data are not shown). The biochemical and histological analysis showed that there were no significant changes in liver and lipid functions nor hepatic or renal toxicity in the treated groups compared with the control group (data are not shown).

3.2 H&E analysis

Figure 2 presents histological sections (5 µm thick) demonstrating the progression of the healing process in the alveolar bone of treated rats. In group 1 (A), there is evidence of immature trabecular woven bone formation, wherein vascular structures are enclosed by woven bone, giving rise to primary osteons. In group 2 (B), secondary osteons can be observed within lamellar bone. The yellow arrow indicates the presence of osteocytes, while the red arrow indicates osteoblasts. The tissue consists of fibrous granulation tissue and mature bone. Notably, a network of immature trabecular woven bone is visible (highlighted by the yellow arrow). group 3 (C) demonstrates the presence of mature bone without fibrous granulation tissue. This group exhibits complete structural units of cortical bone, characterized by the presence of Haversian channels.

3.3 Masson's trichrome analysis

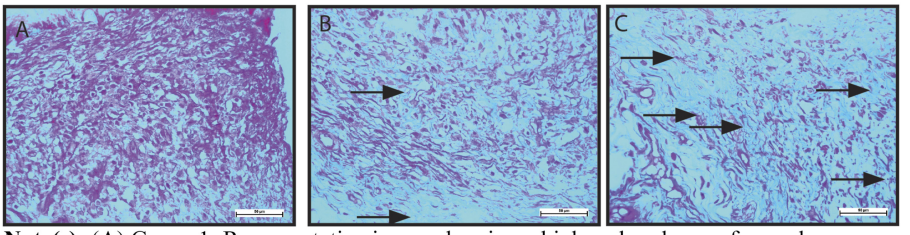
Histological analysis using MT staining revealed the presence of collagen fibers in all experimental groups. Collagen, a prominent constituent of the extracellular matrix within bone, assumes a pivotal function in conferring structural integrity and bolstering the skeletal framework. In group 1 (A), a higher abundance of vascular granulation tissue was noted (Figure 3A). In group 2, a moderate amount of vascular granulation tissue and collagen fibers were observed (Figure 3B). Whereas, the samples in group 3 showed a clear pattern of woven tissue and had mineralized bone tissue with a high level of mineralization, as shown by the strong red stain (Figure 3C).



Note(s): Group 1 (A): histological analysis reveals the presence of immature trabecular woven bone. Vascular structures are surrounded by woven bone, giving rise to primary osteons. Group 2 (B): histological sections show the presence of lamellar bone with secondary osteons. Osteocytes can be observed (indicated by the yellow arrow), along with the presence of osteoblasts (marked by the red arrow). The tissue consists of fibrous granulation tissue and mature bone, indicating an advanced stage of healing. Group 3 (C): histological analysis demonstrates the presence of mature bone without fibrous granulation tissue. Complete structural units of cortical bone are observed, characterized by the presence of Haversian channels. The healing process in this group has progressed to a stage where mature bone formation is prominent. Histological sections stained with hematoxylin and eosin 200X

Source(s): All the figures were made by authors

Figure 2.
Histological sections (5 µm thick) demonstrating the healing process in the alveolar bone of treated rats



Note(s): (A) Group 1: Representative image showing a higher abundance of vascular granulation tissue (blue stain) with minimal collagen fiber deposition (Red stain). (B) Group 2: Representative image demonstrating a moderate amount of vascular granulation tissue (blue stain/Black arrow) and collagen fibers (Red stain). (C) Group 3: Representative image exhibiting a distinct pattern of woven tissue (blue stain/black arrow) and mineralized bone tissue with a high level of mineralization (red stain) Histological sections stained with Masson's trichrome staining 200X
Source(s): All the figures were made by authors

Figure 3.
Histological analysis of the experimental groups using Masson's trichrome (MT) staining revealed the presence of collagen fibers in all groups

3.4 Immunohistochemistry assay

According to Rammelt's (2005) research, it was discovered that OCN has the potential to augment bone remodeling in hydroxyapatite/collagen composites, thereby expediting the process of bone formation and regeneration (Rammelt *et al.*, 2005). On the other hand, previous studies indicated that the origin of the proliferative activity in the healing of extraction wounds in rats can be attributed to the fibroblasts and endothelial cells present in the remaining periodontium (Sato & Takeda, 2007). Furthermore, it has been suggested that the fibroblasts present in the granulation tissue undergo differentiation into osteoblasts, thereby facilitating the formation of fresh bone during the process of socket healing. Thus, our results showed that group 3 demonstrated notable manifestations of osteogenesis as evidenced by the presence of OCN and PCNA at week 4 (refer to Figure 4C/Black arrow), compared to the control group (Figure 4A). These findings suggest that the process of bone maturation was augmented due to the heightened activity of osteoblast proliferation, signifying the differentiation and maturation phase of these cells (Thorwarth *et al.*, 2005).

3.5 Histomorphometric study

Histomorphometric analysis showed a significant difference in the labeling of OCN and PCNA in group 3 compared with other groups (Figure 5). At the beginning of bone mineralization, there was a higher expression of OCN and PCNA in osteoblasts, osteocytes and bone lining cells on week 4 after tooth extraction ($P < 0.05$), specifically in the group 3; in comparison with other groups. This was due to a large number of osteocytes (primarily bone lining cells) in the bone trabeculae' demonstrating the prevalence of OCN and PCNA labeling in these cell types, which led to an accelerated healing process post-tooth extraction through means of enhanced mineralization that was prompted by eggshell treatment.

4. Discussion

Synthetic bone substitutes have been introduced into clinical use, but they are rather expensive. Therefore, avian eggshell powder extracted from animals has been suggested as a possible solution (Durmuş, Celik, Ozturk, Ozkan, & Aydin, 2003). The eggshell membrane contains naturally glycosaminoglycans and proteins, which are essential for bone and connective tissue regeneration.

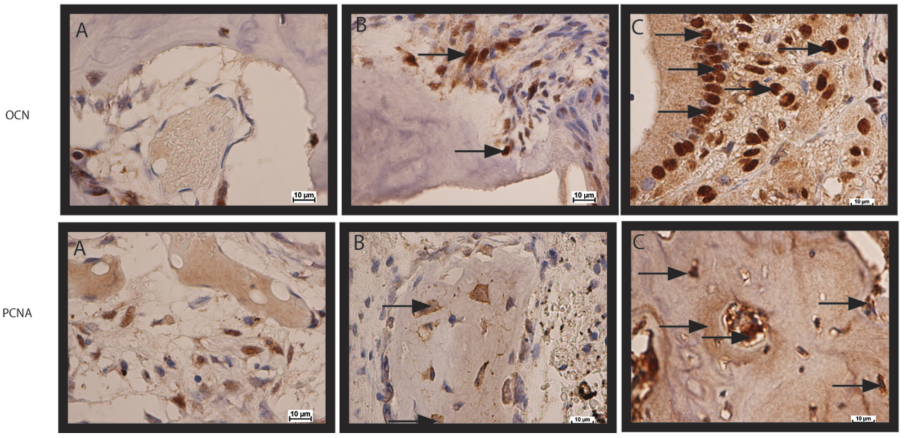


Figure 4.
Immunohistochemistry analysis was performed to evaluate the expression of osteocalcin (OCN and PCNA) in the different experimental groups

Note(s): Group 1 (A): Minimal OCN and PCNA expression were observed in the control group at week 4.: Group 2 (B) demonstrated significant manifestations of osteogenesis, as indicated by the pronounced presence of OCN and PCNA expression (Black arrow). Group 3 (C) The higher expression of OCN and PCNA in this group suggests enhanced bone maturation and osteoblast activity (Black arrow). DAB with haematoxylin/eosin counterstaining, original 20T (OCN = arrow; DAB = diaminobenzidine)
Source(s): All the figures were made by authors

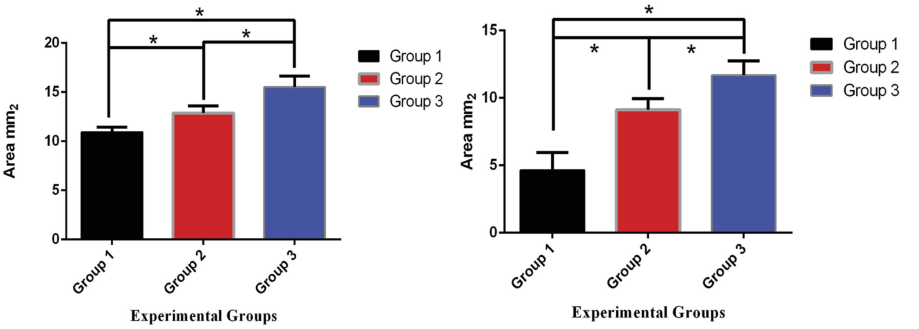


Figure 5.
Mean and standard deviation of OCN (A) and PCNA (B) expression area (mm2) in the extracted teeth for whole experimental groups at 28 days

Note(s): Asterisk (*) indicates significant differences among groups at ($p < 0.05$)
Source(s): All the figures were made by authors

Our results showed that osteoblast and fibroblast cells viability were stimulated by sHAp through the proliferation of both normal cells. Our results are similar to Ruff (Ruff, Endres, Clewell, Szabo, & Schauss, 2012), who depicted that there is no cytotoxicity, genotoxicity, acute and oral toxicity when tested in vitro. The in vivo acute toxicity experiment also showed that no significant changes in animals treated with sHAp, which is similar to the previous results concluded by Vallet Regí (Vallet-Regí, 2001).

Daily mass production of eggshells leads to environmental pollution which was why the HAp used for this study was sourced from recycled eggshells. Bone graft material such as HAp is used in dental applications due to its biocompatibility and has a similar composition to the inorganic extracellular matrix component of hard tissue (Saber-Samandari, Saber-

Samandari, Ghonjizade-Samani, Aghazadeh, & Sadeghi, 2016). Calcium is the main component of HAp obtained from eggshells. It is essential to shrink the impurities like silica and also reduce the cost (Siva Rama Krishna *et al*., 2007). Previous research showed the applications of bioactive calcium phosphate materials in bone regeneration (Jeong, Kim, Shim, Hwang, & Heo, 2019). It is emphasised that the significance of regulating the physical and chemical properties of calcium phosphates to guarantee their efficacy in particular applications. It delves into the topic of calcium phosphates' biocompatibility and the difficulties that come with their usage. These challenges include variations in bone regeneration and degradation rates, restrictions in pore size and inadequate mechanical strength. This information can contribute to the clinical treatment approach for bone defects and diseases. Moreover, another set of findings suggested that the structural and biological properties of β-tricalcium phosphate (β-TCP) ceramics are improved by the inclusion of iron ions (Manchón *et al*., 2015). The incorporation of iron induces modifications in the microstructure and porosity of β-TCP, thereby stimulating the proliferation of osteoblasts and the generation of fresh osseous tissue. The incorporation of iron into calcium phosphate ceramics has been found to improve their osteoconductive properties, thereby expanding the potential applications of iron in bone regeneration therapies. HAp has been shown to have excellent biological properties due to the existence of several ions in it such as Na, K, Mg, Sr, F, Cl and SO4 which potentially aid in the acceleration of bone regeneration (Akram, Ahmed, Shakir, Ibrahim, & Hussain, 2014; Piccirillo *et al*., 2015). The eggshells are conveniently placed at surgical sites due to its hydrophilic character that enables easy absorption by body fluids. HAp is considered the current gold standard as it is thermodynamically stable at physiological pH. It also enhances the alveolar bone by maintaining osteoconductive support during the healing process (Sheikh, Sima, & Glogauer, 2015). It was previously observed that HAp is capable of connecting with the live bone after implantation, despite circumstantial bone defects (Gao *et al*., 2014), subsequently, stem cell proliferation and proper vascularization were also enhanced for excellent guided bone regeneration without any side effects (Yu, Tang, Gohil, & Laurencin, 2015). Previous papers concluded that the use of eggshell-derived nanohydroxyapatite (EnHA) along with the periosteal membrane as a guided tissue regeneration barrier provides substantial evidence for bone regeneration both radiographically and clinically (Vani *et al*., 2023). The combination of periosteal pedicle and EnHA for the treatment of intrabony defects shows promise in the field of regeneration research. In our study, it is revealed that osteoblast adhesion has been stimulated, with bone deposition, which is similar to previous reports (El-Ghannam, Amin, Nasr, & Shama, 2004; Kasaj *et al*., 2008), resulting in rapid bone regeneration due to HAp. In addition, based on the MTT analysis, the results illustrated that collagen fibers were heightened in the treated group (sHAp), which is similar to the previous study that observed collagen fibers were more rapidly generated in the HAp treated group as compared to the control group (Tsuchiya *et al*., 2008). HAp has proven to be viable support that hastens new bone formation by deposition of extracellular matrix (Yu *et al*., 2015) at the site of a bone defect, as previously discussed (LeGeros, 2008).

Our results also demonstrated that OCN is highly expressed in group 3 which was treated with sHAp. OCN is considered the main indicator of the mineralization process which is the late marker for bone formation (Christenson, 1997). The osteoblast is the main producer for OCN (Christenson, 1997). During the differentiation of osteoblast, OCN might be enhanced and secreted for the mineralization process and is an indicator of osteocyte calcification (Calvo, Eyre, & Gundberg, 1996). It is also shown that OCN can be combined into the bone matrix by binding to HAp in a calcium-dependent approach. Similarly, the mineralization process occurred by OCN expression that binds into the bone matrix via linking to HAp (Fang *et al*., 2019), during the alveolar healing process in ovariectomized rats.

Our results also showed that PCNA expression is enhanced in group 3 in comparison to the control group. PCNA is directly correlated with the proliferative state of the cell (Casasco *et al*., 1996). Several studies have shown that PCNA is indicative of osteoprogenitor cells (Ding *et al*., 2013; Pavlidis, Bourauel, Rahimi, Götz, & Jager, 2009). Impaired bone tissue regeneration may be observed due to diminished proliferating cells (Wallner *et al*., 2015). Cell proliferation in normal bone was significantly higher in weeks 1 and 3 in comparison to diseased rats (Colombo *et al*., 2011). It was previously reported that PCNA positives cells were separated among connective tissue treated with HAp microparticles in calvarial critical-sized defect rats, indicating bone formation process (Tour, Wendel, & Tcacencu, 2014). Similarly, a former study has concluded that a significant bone defect treated with HAp was enhanced, confirmed via IHC and histomorphometric analysis coupled with digital image analysis (de Freitas Costa & Neusa Motta, 2009).

The results obtained from this rat model study may inform future clinical trials and potentially contribute to the development of innovative dental treatment options that support new bone formation, enhance socket healing and promote successful implantation of dental prosthetics patient.

5. Conclusion

In conclusion, locally implanted sHAp extracted from eggshells that were used to fill the extracted tooth sockets of rats is an excellent novel bone substitute material. In addition, sHAp might be a perfect osteoconduction-process inducer, as it promotes fibrotic processes by initiating the proliferation of collagen connective tissue and fibroblasts. Thus, bone formation in locally implanted sHAp is more rapid as indicated by the presence of osteoprogenitor cells along the collagen fibers throughout the process of wound healing. Therefore, a comprehensive and methodical review of the existing results on the clinical efficacy of eggshell as a potential substitute material in guided bone regeneration for oral surgery, as applied previously.

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