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Magnesium-calcium/hydroxyapatite (Mg-Ca/HA) composites with enhanced bone differentiation properties for orthopedic applications

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ABSTRACT

Mg-1Ca/HA composites were produced by blending magnesium-calcium (1 wt%) (Mg-1Ca) alloys with 5, 10 and 15 wt% of hydroxyapatite (HA). Morphology, elemental and phase composition of Mg-1Ca/HA composites were examined. Biocompatibility assessments were also performed using an indirect contact method by culturing human adipose mesenchymal stem cells (hASCs) in the extracts of Mg-1Ca alloy, Mg-1Ca/HA composites and Dulbecco's modified eagle's medium. Mg-1Ca/HA composites could promote cell proliferation and at the same time, enhanced collagen type I (COL I) and osteocalcin (OCN) expressions of hASCs. Among the Mg-1Ca/HA composites, 10 wt% of HA is the optimum amount to be added into Mg-1Ca alloy for enhanced bioactivity, thus emerging as a potential biomaterial for orthopedic fixation.

1. Introduction

Biodegradable metals such as magnesium (Mg)-based materials have garnered increasing attention as they possess good mechanical properties, high strength-to-weight ratio, and at the same time, being biodegradable and biocompatible [1–3]. Mg-based materials have higher yield strength in comparison to metals, and their elastic modulus are reported to be similar to that of natural bones [1]. Therefore, Mg-based materials serve to avoid stress-shielding effects [1]. Furthermore, Mg is an essential element in human body, which assists the association of calcified tissue mineralization, and has stimulatory effect on the growing of bone tissues [2,4].

Despite the favorable mechanical properties and biocompatibility, the accelerated corrosion of Mg-based implant surface *in-vivo* is a major concern that deters its usage. Mg corroded at a very high rate in human physiological environment [2]. To reduce the corrosion phenomenon, alloying elements such as calcium (Ca) was added to form Mg-Ca based alloys that contained certain specific phases, which were non-toxic, and were able to provide corrosion resistant properties [2,5].

During the early stage of implantation, it was found that bone response to Mg-Ca based alloys was not improved considerably despite the addition of Ca [6]. In order to enhance the bioactivity of Mg-Ca based alloy, a possible approach is to incorporate hydroxyapatite (HA) to form Mg-Ca/HA composites. HA has a similar chemical and crystallographic structure to bone and thus, could greatly give rise to the superior biological property of improved protein and cell adhesion [7,8]. Therefore, Mg-Ca/HA composite is a potential material for orthopedic applications, and it is the interest of this study to evaluate the addition of different amount of HA to Mg-Ca based alloy for optimum physicochemical and biological properties.

2. Experimental

HA was synthesized via an aqueous precipitation reaction between calcium hydroxide (Merck) and orthophosphoric acid (Merck) according to the method described in our previous study [9], and Mg-Ca (1 wt%) (Mg-1Ca) alloy was produced according to [10]. Different weight percentages of Mg-1Ca alloy and HA (Table 1) were blended via ball milling (ball:sample = 5:1) for 24 h at 24 °C. Subsequently, Mg-1Ca/HA composite powders was compacted uni-axially into ∅13 mm discs before dry heating at 400 °C for 2 h in argon, for the biocompatibility assessments.

Morphology of the sample discs was studied using field emission electron microscopy (HITACHI–SEM6300). The composition and phase purity of sample discs were determined by energy-dispersive X-ray (EDX) spectroscopy and X-ray diffraction (Bruker), respectively.

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Table 1
Weight percentage of Mg-1Ca alloy and HA in various Mg-1Ca/HA composites.

	Mg-1Ca alloy	Mg-1Ca/ 5HA	Mg-1Ca/ 10HA	Mg-1Ca/15HA
HA (wt%)	0	5	10	15
Mg-1Ca alloy (wt%)	100	95	90	85

The biocompatibility assessment was carried out by indirect contact method [3]. Sample discs were sterilized by ultraviolet exposure for 120 min, and were immersed in Dulbecco's modified eagle's medium (DMEM) serum-free medium based on the surface area of extraction medium ratio 1.25 mL/cm^2 for 72 h. The supernatant fluid was centrifuged before being used as the extract medium. Human adipose-derived mesenchymal stem cells (hASCs) isolated from human adipose tissue lipoaspirate (Invitrogen, StemPro® Human Adipose-Derived Stem Cell Kit, Passage 4) were thawed, cultured and maintained in DMEM containing 1% penicillin/streptomycin solution.

3×10^4 cells/mL were incubated in each 96-well at 37 °C in a humidified atmosphere of 95% air and 5% carbon dioxide for 24 h to allow attachment. The medium was then replaced with 100 μL of the respective extract medium. After 7 days, the extract was changed to osteogenic inductive extract, which is the extract medium supplemented with 10 mM of β-glycerolphosphate, 100 μM of ascorbic acid and 10^{-8} M of dexamethasone. Cell proliferation and viability were measured using the alamarBlue™ assay (Invitrogen), and fluorescein diacetate (FDA) and propidium iodide (PI) assays, respectively. The bone cell differentiation was assessed based on the Type I collagen (COL I) and osteocalcin (OCN), as described in our previous study [11]. Statistical significance was considered at $p < 0.05$ based on student t-test.

3. Results and discussion

Fig. 1a and b show the morphology of Mg-1Ca alloy powders and the cross section of compacted Mg-1Ca alloy disc. In both images, Mg-1Ca alloy consisted of spherical particles with an average diameter of 190 ± 30 μm. The surfaces of Mg-1Ca particles became rougher as HA particles were observed to be coated onto the Mg-1Ca particles during the ball milling process (Fig. 1c, e and g). In Mg-1Ca/5HA, some of the Mg-1Ca particles were partially coated with HA, and this was further shown in the cross section of the compacted Mg-1Ca/5HA disc (Fig. 1d). However, when the amount of HA was increased to 10 wt% and beyond, all the Mg-1Ca particles were coated with a layer of HA (Fig. 1f). Nevertheless, excessive HA particles were seen coated onto the Mg-1Ca particles in compacted Mg-1Ca/15HA disc (Fig. 1h).

Mg and Ca were detected for Mg-1Ca alloy whilst Mg, Ca and P were detected for all Mg-1Ca/HA composites (Fig. 2a). There was no major change in the weight percentages of Mg-1Ca alloy and HA of the Mg-1Ca/HA composites after heat treatment. The detection of P indicated the presence of apatite in Mg-1Ca/HA composites. Furthermore, the increasing intensities of Ca and P peaks from Mg-1Ca/5HA to Mg-1Ca/15HA suggested the increasing amount of HA in the Mg-1Ca/HA composites. The XRD patterns of Mg-1Ca alloy, and all Mg-1Ca/HA composites exhibited dominant α-Mg reflections (pdf no. 350821) in the range from 30° to 50° (Fig. 2b). In addition, there were also evident peaks for apatite phase, detected at 31.7 and 32.9° in all Mg-1Ca/HA composites. The low amount of HA in Mg-1Ca/HA composites gave rise to relatively low intensity of apatite as compared to Mg. However, it was noted that the relative intensity of apatite at 31.7 and 32.9° increased from Mg-1Ca/5HA to Mg-1Ca/15HA, which corresponded to the amount of HA in the Mg-1Ca/HA composites. This was in line with the EDX results with regards to the increasing amount of HA in the Mg-1Ca/HA composites. On the other hand, these two peaks were not detected in Mg-1Ca alloy. Overall, the results of XRD analysis demonstrated that no other secondary phases such as Mg_2Ca was detected in all samples, implying that there were no reaction between the Mg-1Ca alloy and HA. The absence of such intermetallic phase was favorable as it could cause embrittlement and weaken the interfacial bonding of particle/matrix, which would in turn affect the mechanical properties [12].

Fig. 3a shows the live/dead staining of hASCs cultured with extracts of Mg-1Ca alloy, various Mg-1Ca/HA composites and DMEM. Cells were all viable when cultured in the extract of Mg-1Ca alloy, various Mg-1Ca/HA composites and DMEM. These viable cells were further quantified using alamarBlue™ assay (Fig. 3b). hASCs cultured in all extracts increased from day 1 to day 5. These results agreed with the previous observation of Li et al. [5], which indicated the biocompatibility of Mg-1Ca/HA composites. With the addition of HA, Mg-1Ca/HA composites showed better bone differentiation effect in contrast to Mg-1Ca alloy and DMEM. This was exemplified when the amount of HA increased to 10 wt% since the expression of COL I and OCN for hASCs cultured in the extract of Mg-1Ca/10HA increased significantly (Fig. 3c and d). It might be due to the solubilization of HA, that subsequently lead to the precipitation of biological apatite layer onto the surface of the biomaterial, which could induce the adsorption of proteins and other bio-organic compounds that further stimulate cell adhesion, proliferation and differentiation [13,14]. On the contrary, the expression of COL I and OCN for the hMSCs cultured in the extract of Mg-1Ca/15HA were lesser than Mg-1Ca/10HA. This effect might be due to the release of excessive calcium ions, which could increase the pH of the extract, thus affecting the bone differentiation. A reduced cell viability was also observed in the study of Feng et al. [3] when a higher Ca content of Mg/Ca composite was cultured with murine fibroblast L-929 cells. Thus, the addition of 10 wt% of HA was suggested to be the optimum amount to promote the bioactivity of the material.

4. Conclusions

Mg-1Ca alloys were blended with 5, 10 and 15 wt% of HA to form Mg-1Ca/HA composites. HA particles were coated onto the Mg-1Ca spherical particles in all Mg-1Ca/HA composites. Phase-pure α-Mg and apatite were found in all Mg-1Ca/HA composites. hASCs cultured in the extracts of Mg-1Ca alloy and all Mg-1Ca/HA composites increased with culturing period, with good cell viability. Among the Mg-1Ca/HA composites, hASCs cultured in the extract of Mg-1Ca/10HA demonstrated enhanced COL I and OCN expressions. All these results suggested that Mg-1Ca/HA composites could enhance the differentiation of hASCs into osteoblasts, and 10 wt% of HA was the optimum amount to be added with Mg-1Ca alloy for enhanced bioactivity.

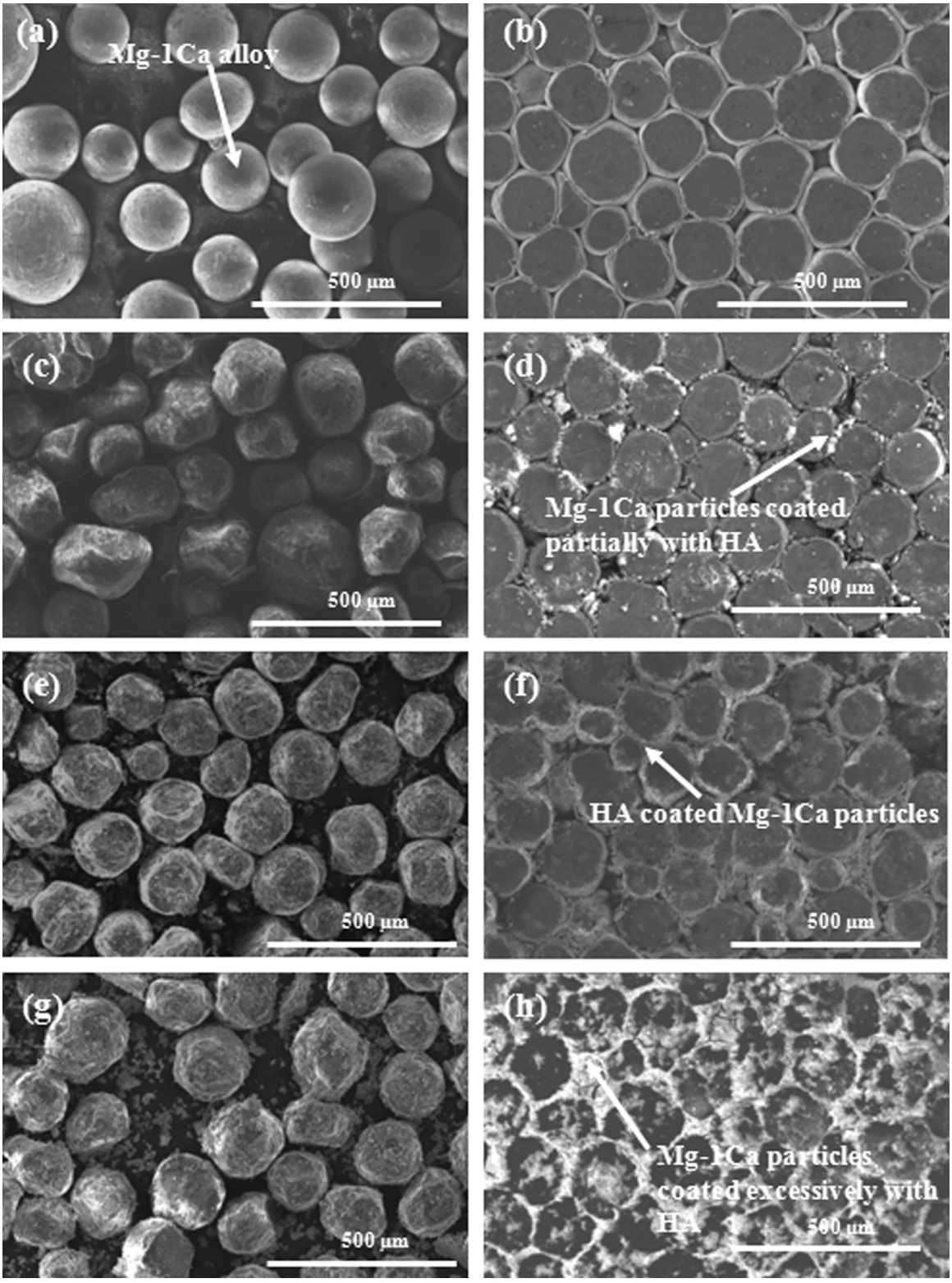

Fig. 1. FESEM images of (a) Mg-1Ca alloy powders, (b) cross section of compacted Mg-1Ca alloy disc, (c) ball-milled Mg-1Ca/5HA powders, (d) cross section of compacted Mg-1Ca/5HA disc, (e) ball-milled Mg-1Ca/10HA powders, (f) cross section of compacted Mg-1Ca/10HA disc, (g) ball-milled Mg-1Ca/15HA powders, and (h) cross section of compacted Mg-1Ca/15HA disc.

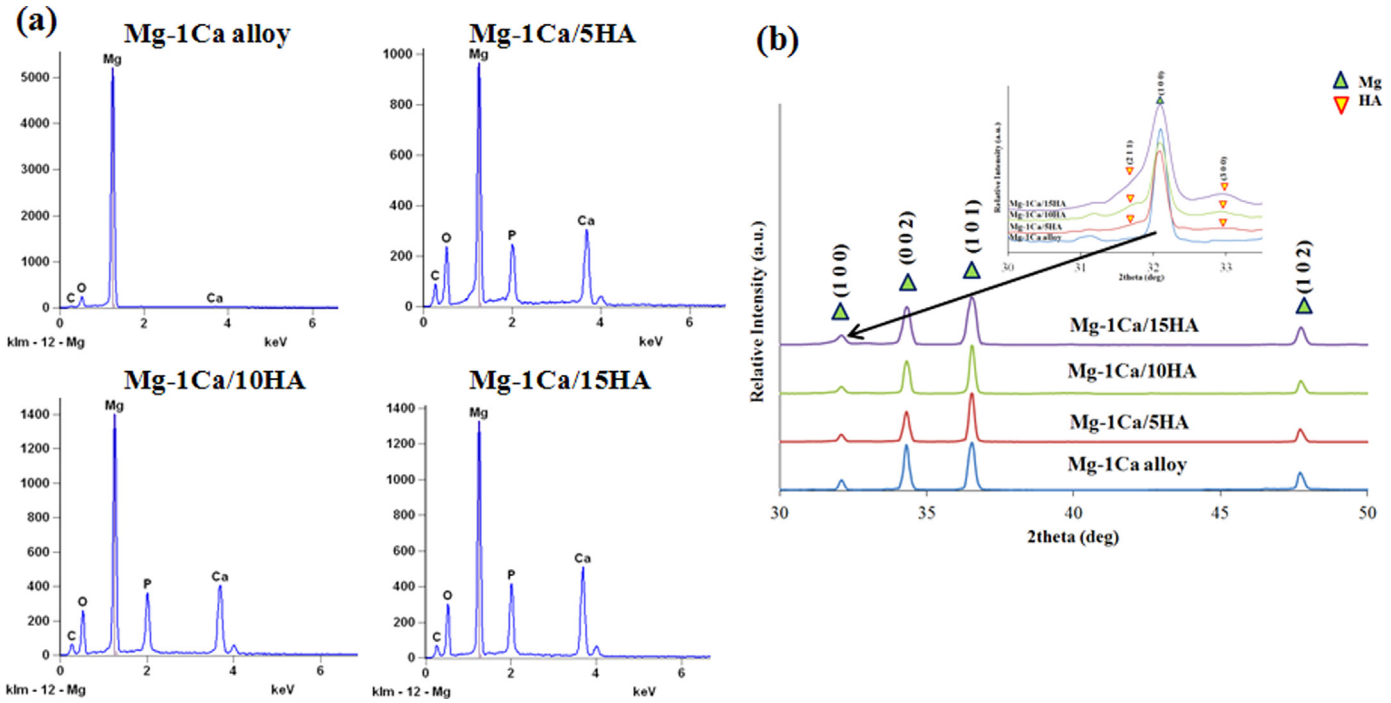

Fig. 2. (a) EDX analysis and (b) XRD patterns of Mg-1Ca alloy and Mg-1Ca/HA composites.

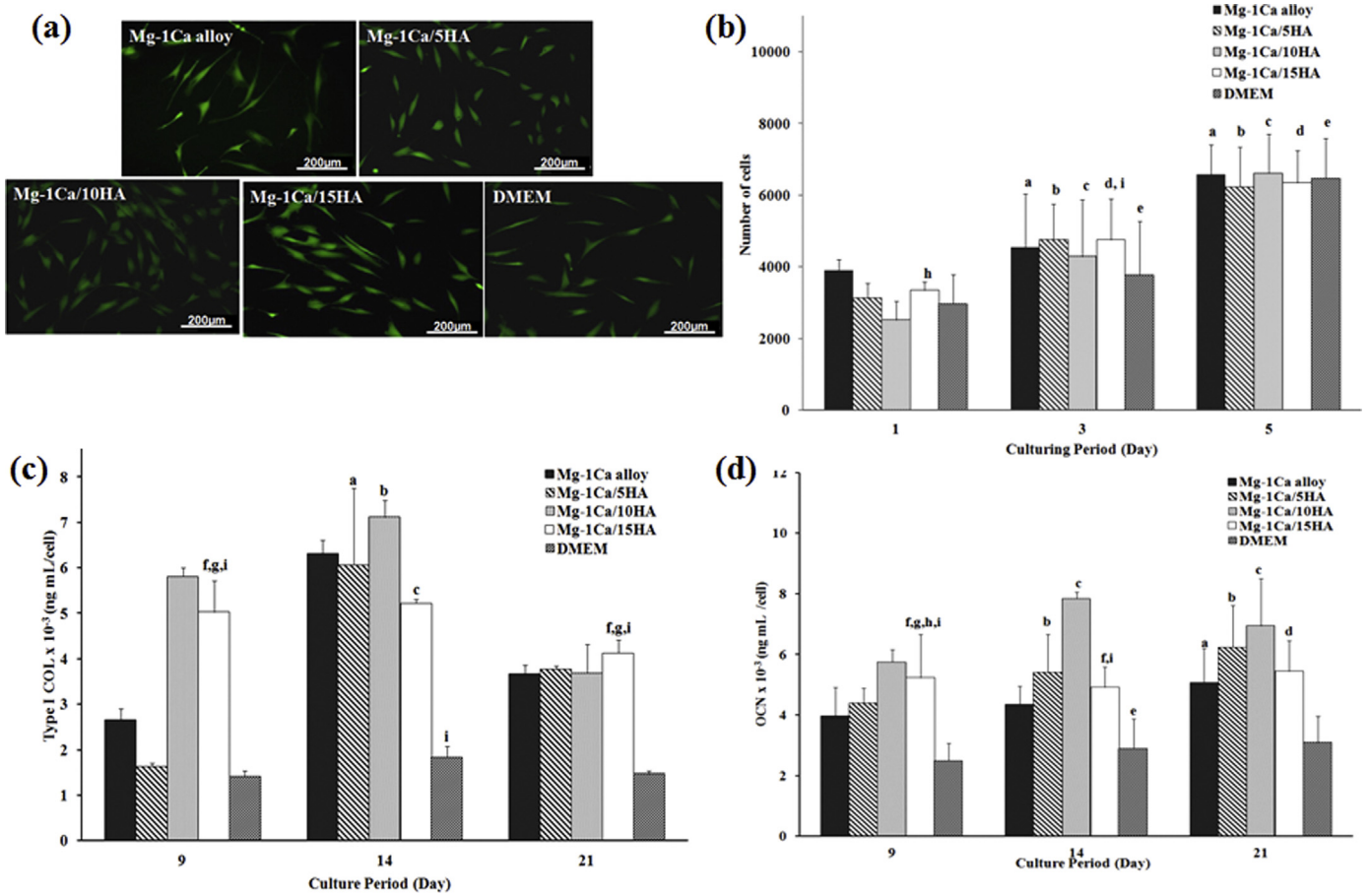

Fig. 3. (a) Cell viability at day 5, (b) cell proliferation, (c) COL I expressions and (d) OCN expressions of hASCs cultured with the extract of Mg-1Ca alloy, Mg-1Ca/5HA, Mg-1Ca/10HA and Mg-1Ca/15HA composites [a] $p < 0.05$ for Mg-1Ca alloy between groups; [b] $p < 0.05$ for Mg-1Ca/5HA between groups; [c] $p < 0.05$ for Mg-1Ca/10HA between groups; [d] $p < 0.05$ for Mg-1Ca/15HA between groups; [e] $p < 0.05$ for DMEM between groups; [f] $p < 0.05$ when comparing Mg-1Ca/15HA to Mg-1Ca alloy within groups; [g] $p < 0.05$ when comparing Mg-1Ca/15HA to Mg-1Ca/5HA within groups; [h] $p < 0.05$ when comparing Mg-1Ca/15HA to Mg-1Ca/10HA within groups; [i] $p < 0.05$ when comparing Mg-1Ca/15HA to DMEM within groups.

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