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Stimulated osteoblastic proliferation by mesoporous silica xerogel with high specific surface area

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Abstract Specific surface area is a critical parameter of mesoporous silica-based biomaterials, however, little is known about its effects on osteoblast responses in vitro. In the present study, mesoporous silica xerogels (MSXs) with different surface area (401, 647 and 810 m^2/g, respectively) were synthesized by a sol–gel process. Surface silanol contents decreased with the increase of surface area with which protein adsorption capability positively correlated. And the apatite-like surface seemed to form faster on MSXs with higher surface area determined by XRD analysis. Using MG63 osteoblast-like cells as models, it was found that cell proliferations were promoted on MSXs with higher surface area, based on the premise that the effects of Si released from materials on osteoblast viability were excluded by real-time Transwell® assay. RT-PCR results indicated cell adhesion-related integrin subunits α5 were up-regulated by higher surface area at day 1, which was further confirmed by flow cytometry analysis. The data suggest that increasing SSA of MSXs could promote surface cellular affinity by adsorbing serum proteins and accelerating apatite-like layer formation, which results in promoted osteoblastic proliferation via integrin subunit α5 at initial adhesion stage. Regulating SSA, an effective approach in designing mesoporous silica-based materials, provides an alternative method to obtain desirable tissue-response in bone regeneration and drug-delivery system.

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1 Introduction

There are an increasing number of people who suffer from traumas, accidents or diseases associated with bones, which stimulate the demand for bone repair [1]. In order to accelerate bone regeneration and reduce the suffering of patients, implantable drug-delivery systems for bone tissue attract great attentions as the most promising therapeutic concept in orthopaedic surgery [2]. In recent years, biocompatible silica-based materials, termed silica xerogels, have shown desirable bioactivity and degradation viability in previous reports both in vitro and in vivo [3, 4]. Mesoporous silica xerogels (MSXs), developed from traditional sol–gel silica xerogels, have formed a subject of intensive research [5, 6]. Their unique properties including stable mesoporous structure, tunable pore size, high specific surface area (SSA), and well modifiable surface, endow them with great potential applications in bone tissue engineering for controlled-releasing antibiotics [7], growth factors [8], anti-inflammatory drugs [9], etc.

The drug-loading process depends primarily on the adsorptive properties based on its mesoporous structure. Therefore, surface becomes the most determinant factor during the process of adsorbing drugs or proteins. It is unquestionable that high SSA of matrix can host large amounts of pharmaceuticals or, at least, incorporate tunable dose of drugs [10]. As long as the pore size allows the drug to get into the matrix, the higher the SSA is, the larger the amount of drugs can be adsorbed. Consequently, the adsorbed drug content is very sensitive to SSA.

When a biomaterial was implanted, successful incorporation of an implant into the body depends on tissue integration influenced by the adherence of cells to the surface. Certain protein will be adsorbed on to the surface soon after surgery, which will mediate cell attachment and biological response [11]. Surface properties of the implants are early recognized to be an important factor during osseointegration. To date, treatments on surface properties, such as surface energy, wettability, surface charge, surface chemical composition, surface roughness and topography do have pronounced impacts on osteoblast morphology, adhesion, proliferation, alkaline phosphatase (ALP) activity as well as regulated signaling expressions [12–14]. All surface modifications could introduce stress to the surface and alter the surface chemistry, which in turn influences surface free energy and ultimate cell responses [15]. However, the biological response of osteoblasts to MSXs with different SSA, an essential parameter for designing mesoporous silica materials, has not been elucidated yet to the present. Herein, the principal purpose of our current study was designed to clarify the potential regulatory role of SSA on the surface properties of MSXs and osteoblast responses, the possible mechanism, and signaling pathway involved during adhesion-related integrins subunits expression.

2 Materials and methods

2.1 Preparation of MSXs with different SSA

MSXs were synthesized according to protocols published previously elsewhere [16]. In brief, cetyltrimethylammonium bromide (CTAB) was first added to deionized water under stirring at room temperature, and when the solution turned to clear, tetraethyl orthosilicate (TEOS) was added under vigorous stirring to obtain a homogeneous solution. Aqueous ammonia was then added to the resulting solution in an appropriate proportion as catalyst. Ammonia/silica molar ratios were adjusted according to parameters shown in Table 1. The mixtures were stirred continuously for several minutes, and then allowed to stand still until the gel was formed, followed by 5 h aging at room temperature. The resulting silica wet gels were washed for three times with distilled water in centrifuge, and dried in air at 70°C overnight, to give the surfactant-contained silica xerogels. To remove the CTAB template, the silica xerogels were ground into fine powder (100–200 μm) and calcined in the air at 1°C/min to 600°C. The resulting samples were stored in desiccators for further characterization.

The morphologies of the samples were observed by scanning electronic microscope (SEM, JSM-6360LV, JEOL, Japan) and transmission electronic microscope (TEM, JEM2010, JEOL, Japan). And SSA was determined by N_2 adsorption–desorption analysis (Tristar 3000, USA).

2.2 Surface bioactivity of MSXs in vitro

The pH variations and the ions released from the gels in simulated body fluid (SBF) were analyzed through the following procedures. Granules of MSXs were suspended in sealed polyethylene bottles containing 20 ml SBF for each sample, yielding concentration of 2% (W/V). After constant agitation for 24 h at 37°C with constant agitation, the granules were removed and the pH value of SBF was determined by pH meter (FE20, Mettler Toleto Co.), and the amount of Si released to SBF was analyzed by an inductively coupled plasma atomic emission spectrometer (ICP-AES, Perkin-Elmer Optima 2000). X-ray diffraction (XRD, Rigaku Co., Japan) was also performed to trace the surface composition changes in the course of immergence on diffractometer with Cu Kα radiation and Ni filter ($\lambda = 1.5406$ Å, 100 mA, 40 kV).

2.3 Determination of silanol content on the surface of MSXs

Thermogravimetry (TG) is a simple and convenient physical method well established before [17]. The mechanism is to measure water weight loss during the heating progress. In our experiment, samples were heated at a rate of 10°C min^{-1} to 1250°C in an argon flow of 100 ml min^{-1}. The condensation of silanol occurs through the reaction of two silanol groups on the silica surface, resulting in release of one molecule of water and formation of one siloxane group. The silinol group, measured in terms of moles of hydroxyls left per gram of silica, can be calculated as follows:

Table 1 Synthetic conditions of MSXs and variations in pH value and Si release after immersion in SBF for 1 day

Series	NH_3:SiO_2 (Molar ratio)	SSA (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)	pH	Si release (ppm)
MSX_{401}	0.06	401	0.58	2.1	6.68	175
MSX_{647}	0.13	647	0.66	2.4	7.25	150
MSX_{810}	0.89	810	0.44	2.2	7.20	77

$$n_{\mathrm{Si-OH}} = 2n_{\mathrm{H_20}} = \frac{2(\mathrm{W}_{(\mathrm{T_0})} - \mathrm{W}_{(\mathrm{T_e})})}{100\mathrm{M}_{\mathrm{H_20}}}$$

$$\alpha_{\mathrm{Si-OH}} = \frac{n_{\mathrm{Si-OH}}}{\mathrm{SSA}}$$

where T_0 and T_e were defined as the temperature right before and after the dehydration process, respectively; $W_{(T0)}$, $W_{(Te)}$ were defined as the weight of samples at T_0 and T_e, respectively. M_{H2O} represents the molar mass of water. n_{Si-OH} and α_{Si-OH} stand for the amount of silanol groups on the surface (in terms of mmol per gram, μmol per m^2, respectively).

2.4 Analysis of serum protein adsorption by BCA assay

Bicinchoninic acid (BCA) assay is one of the colorimetric protein assay techniques detecting the cuprous ions generated from cupric ions through reaction with protein under alkaline conditions [18]. MSXs were soaked into Dulbecco's minimum essential medium (DMEM, GIBCO, Grand Island, NY) with 10% fetal bovine serum (FBS, Sijiqing, Hangzhou, China) at a final concentration of 1% (W/V). After incubation for 2 h at 37°C, the culture medium was aspirated and the materials were washed with cold phosphate buffered solution (PBS) and centrifuged for three times. The materials were then washed with 1% sodium dodecyl sulfate (SDS) solution and centrifuged for three cycles to extract the proteins into the SDS solution. The amount of protein in these extractions was determined by a commercially available BCA assay kit (Beyotime biotech, Jiangsu, China).

2.5 Cell culture

At 37°C in a humidified 5% CO_2 atmosphere, MG63 osteoblast-like cells were cultured in DMEM supplemented with 10% FBS plus 100 U/ml penicillin and 100 μg/ml streptomycin sulfate. Cells were incubated in 25 cm^2 flasks to reach 80% confluence and then detached for further experiments. Prior to being seeded onto, MSXs were sterilized by dry heat at 200°C for 30 min and placed in tissue culture plates.

2.6 Cell proliferation assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a colorimetric assay that is based on measuring changes in absorbance at a specific wavelength and is widely used for measuring cytotoxicity and proliferation. Cell density was determined using counting chamber and adjusted to 2×10^4/ml. The cells were cultured on MSXs for 3 days, after which an MTT (MajorBiochem, Shanghai, China) solution (5 mg/ml) was added to give purple formazan in osteoblast cultures on MSXs. The culture medium was aspirated, followed by addition of dimethyl sulfoxide (DMSO) for 10 min at 37°C. One hundred micro litre solutions were then transferred to 96-well ELISA plate and the absorbance was measured at 492 nm using a microplate reader (MULTISKAN MK3, Thermo Electron Co., USA).

Real-time ion release (Transwell®) assay: Transwell® (Costar, Corning) was used in this experiment, which contains an insert. After cultured for 3 days, inserts were removed and effect of ions released from MSXs on osteoblasts growth was determined by MTT assay as described above.

2.7 RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Gene expression was analyzed using reverse transcriptase polymerase chain reaction (RT-PCR). Cells were cultured in 24-well tissue culture plates in the presence of MSXs for 24 h. Total RNA in the cultures was extracted using an AxyPrep™ total RNA purification kit and a purification column (Axygen, Qiagen, Valencia, CA) in accordance with manufacture's protocols. Following DNAse treatment, reverse transcription of 2 μg of total RNA was performed using MMLV reverse transcriptase (Promega, USA) in the presence of oligo$(dT)_{18}$ primer (Takara, Japan). PCR was performed using *Taq* DNA polymerase kit (Tiangen Biotech, Beijing, China) to detect integrin subunits α1, α2, α5 using the primer designed (showed in Table 2) and PCR conditions are following: an initial denaturation of 10 min at 94°C was followed by 28–32 cycles of 45 s at 94°C, 45 s at variable Tm temperature, and 1 min at 72°C, followed by 10 min of final elongation at 72°C. PCR products were visualized on 1–3% agarose gel with Goldview™ (SBS Biotech, Shanghai) staining. Band intensity was detected under UV light and normalized with reference to GAPDH.

2.8 Detection of integrin α5 expressions by flow cytometry

After incubation for 24 h, cells were detached from the materials after washed with 50 mM EDTA in PBS, centrifuged and resuspended in PBS containing 1% BSA. The cells were then incubated with integrin α5 antibodies (BD biotech, NJ) for 30 min at 4°C. After washed with PBS containing 1% BSA, cells were incubated with FITC-conjugated secondary antibodies for 30 min at 4°C and then analyzed by flow cytometry (FACS Calibur, Bector Dickinson), collecting 10,000 events.

Table 2 Primers utilized for RT-PCR amplification

Gene	Primer	Sequence	Tm (°C)	Products (bp)
Integrin α1	Sense	5′-TGCCAGTGAGATTTCAGAGACC-3′	60	117
	Anti-sense	5′-GTGATTTCCTGTGTTTTCGTCG-3′		
Integrin α2	Sense	5′-AACTCTTTGGATTTGCGTGTG-3′	58	82
	Anti-sense	5′-TGGCAGTCTCAGAATAGGCTTC-3′		
Integrin α5	Sense	5′-TGCCTCCCTCACCATCTTC-3′	55	482
	Anti-sense	5′-TGCTTCTGCCAGTCCAGC-3′		
GAPDH	Sense	5′-CCATGGAGAAGGCTGGGG-3′	55	194
	Anti-sense	5′-CAAAGTTGTCATGGATGACC-3′		

2.9 Statistical analysis

The number of samples was four parallel for culture experiments. One-way ANOVA analysis with Tukey's post hoc test was used to examine the variance between the cultures with different MSXs. A P value of <0.05 was considered statistically significant.

3 Results

3.1 The surface characterization of MSXs

Three types of mesoporous silica xerogels, with different SSA (401, 647 and 810 m^2/g) were prepared by varying the molar ratio of ammonia/silica (Table 1). TEM image shown in Fig. 1 provided further direct observations on the mesoporous framework structure of all three MSXs, which contained randomly distributed wormhole-like mesoporous channels with similar pore size. With the increase of SSA, clearer mesopore structure in MSXs could be observed.

Variations in the pH value and the concentration of ions released from MSXs which might influence their responses to osteoblast functions were determined by immersing them in SBF for 24 h, respectively, as shown in Table 1. The pH value changed within the range of 6.6–7.3, which should not be harmful to cells viability. The pH value was elevated in the MSXs with higher SSA. A slight decrease of pH after immersion might result from dissolution of silicic acid.

3.2 Apatite formation and bioactivity in SBF

Figure 2 presents the XRD data on MSXs surfaces after immersed in SBF for different periods. The peak assignments in the XRD spectrum were done according to the articles published previously [19]. MSXs were non-crystallized materials without specific peaks in XRD patterns (as shown at 0 h for all MSXs in Fig. 2). The sharp peaks at $\sim 31.5°$ and $\sim 45°$, characteristic of apatite crystals, first started to form in MSX_{810} after incubation for 1 h. The same peaks became detectable in MSX_{647} after incubation for 12 h. These peaks in MSX_{401} were finally detected after incubation for 72 h.

3.3 Effect of SSA on surface silanol content of MSXs

Using thermogravimetry (TG) method, we measured the n_{Si-OH} and α_{Si-OH} values for three samples of MSXs (Fig. 3). From the results in Fig. 4, it is evident that

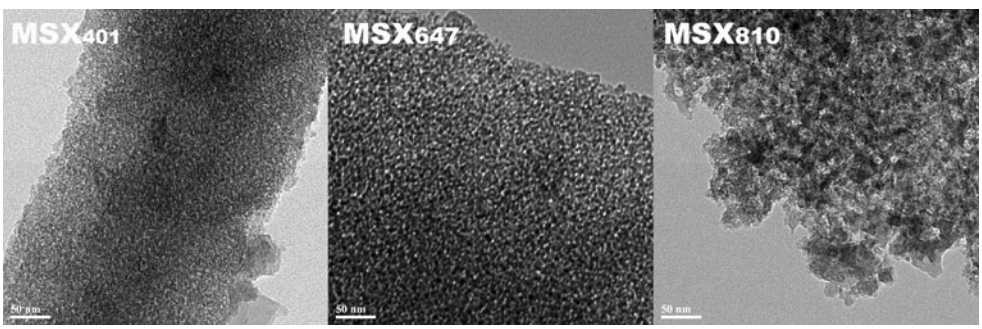

Fig. 1 Typical surface morphologies of MSXs taken by TEM

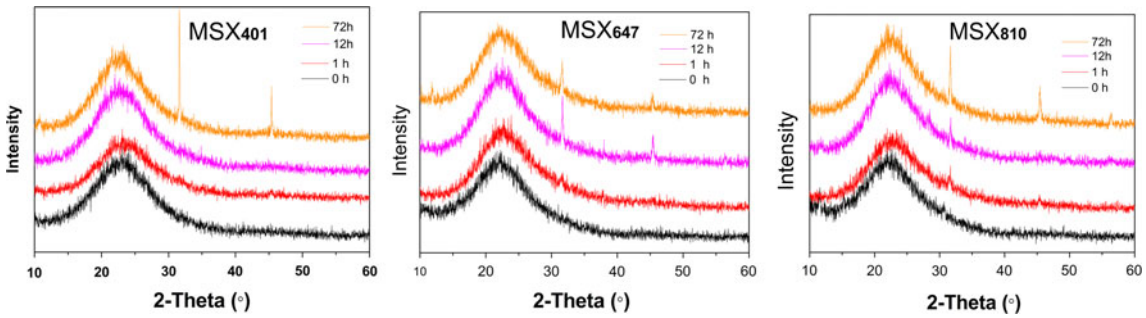

Fig. 2 Determination of surface bioactivity of MSXs. XRD patterns of MSXs with different surface area after immersion in SBF for different periods of time (0, 1, 12, 72 h)

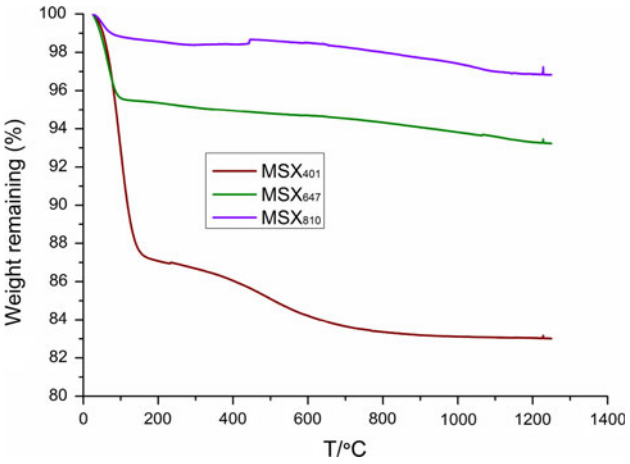

Fig. 3 Determination of silanol content on the surface of MSXs with different SSA by thermogravimetry (TG) analysis

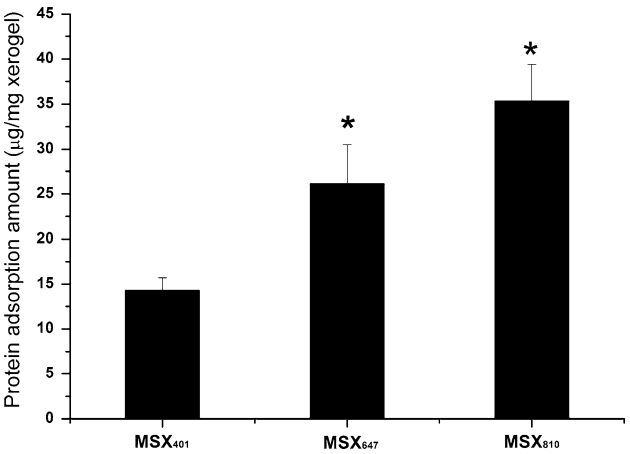

Fig. 4 Protein adsorption of MSXs with different SSA. MSXs were immersed in DMEM + 10% FBS culture medium for 2 h at 37°C, and then protein adsorbed was extracted by SDS and determined by BCA assay. *$P < 0.001$, compared with MSX_{401}

MSX_{401} had the highest value of surface –OH groups of 6.42 mmol/g and 16.0 μmol/m^2 among all three samples. With increasing SSA of MSXs, the values of $n_{Si–OH}$ decreased to ~41 and ~33% in MSX_{647} and MSX_{810}, respectively (Table 3). The data of $\alpha_{Si–OH}$ also showed significant decrease with higher SSA.

Table 3 The hydroxyl group content on surface of MSXs determined by TG analysis

*n*Group	MSX_{401}	MSX_{647}	MSX_{810}
$n_{Si–OH}$ (mmol/g)	6.42	2.65	2.10
$\alpha_{Si–OH}$ (μmol/m^2)	16.0	4.09	2.59

3.4 Effect of SSA of MSXs on protein adsorption

Serum protein adsorption was determined by BCA kit. Figure 4 displayed results for total protein adsorbed by MSXs. MSX_{810} and MSX_{647} held much more protein (up to 35.3 and 26.1 μg/mg xerogel, respectively) than MSX_{401} (14.3 μg/mg xerogel) ($P < 0.001$). After linear regression analysis, it was also observed that the protein adsorption was positively related to SSA, which follows equation: PA = 0.0511 SSA-6.39, $R^2 = 0.9958$, where PA stands for protein adsorbed (μg/mg xerogel).

3.5 Effect of SSA of MSXs on osteoblasts proliferation

MTT confirmed that metabolism of MG63 was affected in the presence of MSXs with varied SSA (Fig. 5a). Cell proliferation was promoted with MSX with increasing SSA at 1 and 2% usage. The OD obtained in MSX_{810} at 1% was ~40% higher than that in MSX_{401} ($P < 0.05$).

The effects of SSA of MSXs on cell proliferation were investigated before and after pre-adsorbing proteins from medium. As shown in Fig. 5b, for MG63 cells, proliferation accelerated after protein adsorbed, and significant difference was observed for MSX_{810} ($P < 0.05$), which indicated that the pre-adsorbed protein promoted the bioactivity of MSX with higher SSA.

To testify the effect of Si ions released from MSXs on cell proliferation, real-time ion release equipment was utilized (Fig. 5c). Under this indirect culture way, the result showed that no significant effect on osteoblast growth,

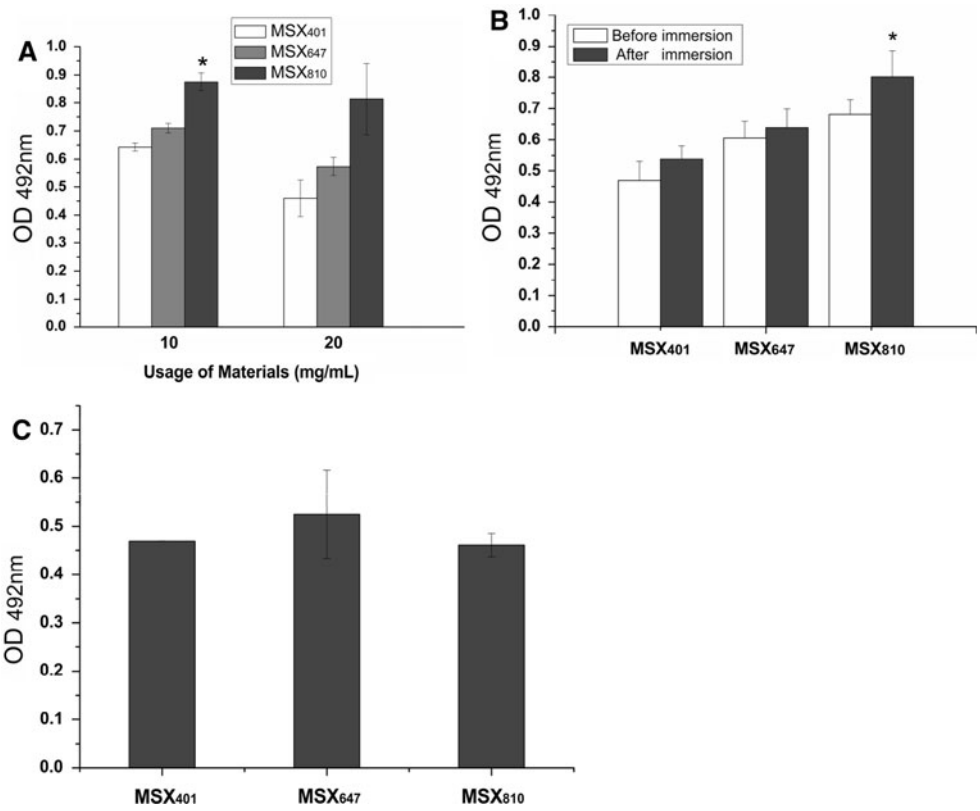

Fig. 5 Enhanced proliferation of osteoblasts by MSXs with higher SSA. **a** Direct contact of MG63 on MSXs at 1 and 2% (W/V), $*P < 0.05$, compared with MSX401; **b** cells were cultured on MSXs before or after pre-immersion, $*P < 0.05$, compared with same MSX before and after immersion; **c** cells were incubated using Transwell® indirect culture. Results are the mean ± standard deviation ($n = 4$)

which was totally different from that under the direct culture system (Fig. 5a). Therefore, the stimulating effect in cell proliferation by ion released from MSXs in the direct way might be excluded.

3.6 Effect of SSA of MSXs on integrin subunits expression

Integrin α1, α2 and α5 expression in osteoblast was evaluated by RT-PCR analysis after cultured on MSXs with different SSA for 1 day (Fig. 6a). Integrin α2 expressions were down-regulated with increasing SSA, whereas α5 expressions were significantly enhanced in osteoblasts. However, no evident variation was observed for integrin α1.

To further investigate cells surface integrin α5 expression, flow cytometry was performed (Fig. 6b). The level of integrin α5 was quantitatively determined by green fluorescence emitted from FITC-labeled antigen on cells. The logarithmic fluorescence intensity of untreated cells was set between 10^0 and 10^2 (as shown in control panel), hence

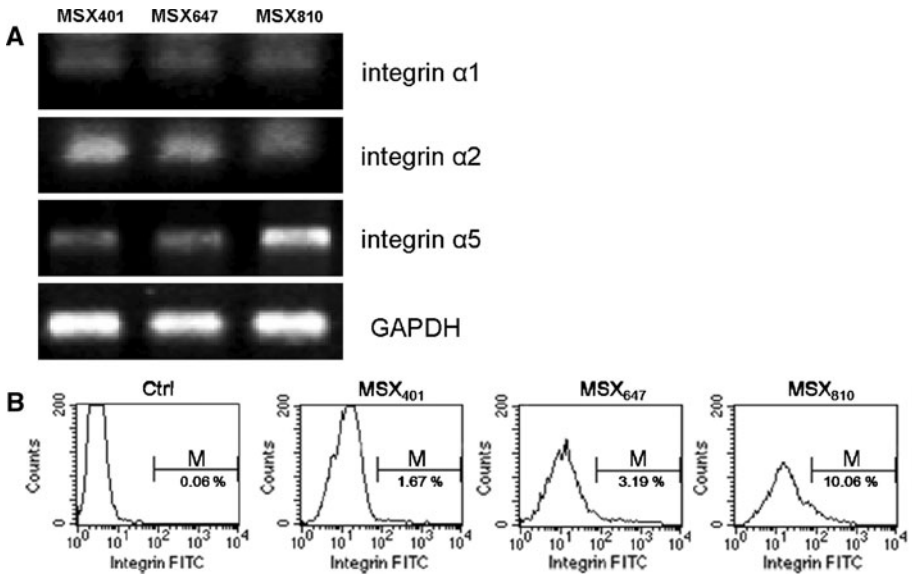

Fig. 6 Integrin α5 was up-regulated by MSXs with higher SSA. **a** RT-PCR for integrin α1, α2, α5 expression. GAPDH, was run as control. **b** Flow cytometry detecting integrin α5 expressions. The number of positively-labeled cells was represented as the percentage of total counting cells in each panel (M). Data shown here are representative from three independent experiments

cells exhibiting green fluorescence intensity higher than 10^2 were considered as positively-labeled cells with integrin α5 expressed (Ctrl panel). The expression level of integrin α5 for MSX_{401} was only about 1.6%, whereas it reached ~3.1% for MSX_{647}. The highest level was observed for MSX_{810}, reaching ~10%, which indicates protein synthesis of integrin α5 in osteoblasts was up-regulated when cultured on MSXs with higher SSA.

4 Discussion

While some studies have demonstrated that chemical composition of biomaterials is important and may result in marked changes in cell responses, physical properties and surface morphology of biomaterials are also critical [20–25]. Mesoporous silica xerogel, a kind of materials with simple chemical composition (SiO_2 in framework), but rather large SSA and unique mesoporous structure [26], shows great potentials in bone tissue engineering advocated to release adsorbed drugs or growth factors in a controlled manner. Herein, the effects of its surface morphology on the osteoblast responses were investigated in this study. By varying the ammonia/silica molar ratio, a series of MSXs with gradient SSA were synthesized via sol–gel process using CTAB as template under basic conditions [16]. Surface morphology and mesoporous structure of the materials were characterized via TEM, SEM, and BET-N_2 gas adsorption. The SSA of MSXs that we used in the present study was determined to be 401, 647 and 810 m^2/g, respectively.

From our results, the proliferation of MG63 was promoted evidently with increasing SSA and the pre-adsorbed protein in culture medium could further enhance it (Fig. 5). Silicon (Si), as an essential element in animal tissues and organs nutrition, plays an important role in local factor secretion, protein and DNA synthesis as well as bone formation and metabolism [27]. The traditional viewpoint of Si ion on the promotion effect of Si on cell proliferation was excluded by real-time Transwell® assay we established. In this system, the membrane on the bottom of the insert allows free transportation of nutrition and ions to the main well. Cells were pre-cultured on the main well and xerogels were located into the insert of Transwell® so that ions released from materials can permeate the membrane and the influence of ions transported on osteoblasts proliferation can be determined. No direct contacts existed between cells and materials. The result might indicate that the amount of Si released was not large enough to stimulate positive cell responses [28].

Cells recognize and adhere to biomaterial surfaces that adsorb proteins non-specifically via integrin receptors. Integrin is a widely expressed family of heterodimeric (α β subunit) transmembrane receptors that provide anchorage forces and trigger signals regulating cell functions. It has been demonstrated that MG63 osteoblast-like cells express α1β1, α2β1, α5β1 integrin [29]. Integrin α1β1 may induce cell proliferation and reduce collagen synthesis, while α2β1 integrin increases matrix remodeling by regulating type I collagen and collagenase gene expression [30]. Essential for bone morphogenesis, integrin α5β1 is a classical fibronectin receptor that mediates critical interactions between osteoblasts and fibronectin [31]. In the present study, it was observed that with increasing SSA of MSXs, the α5 expression was up-regulated in osteoblasts significantly as demonstrated by RT-PCR and flow cytometry. It is rational that the discrepancy in cell proliferation might result from specific adhesion signaling pathway when cells interacted with MSXs bioactive surface. The promotion in osteoblast proliferation might result from higher interaction/binding efficiency between certain adhesion molecules, such as fibronectin ligands and α5β1 integrins when osteoblasts adhered to MSXs with higher SSA.

In terms of xerogels surface modifications, there are two critical factors yet to be addressed. The first one is the large protein adsorption capability that changes surface affinity of mesoporous materials [32]. The second one is the surface bioactivity, which has been recognized as a crucial aspect when osteoblasts grow and differentiate on certain matrix.

In our study, it is confirmed by BCA assay that the amount of adsorbed serum protein increased with SSA (Fig. 4). The pre-adsorbed protein on xerogels further facilitated osteoblast proliferation. When materials with mesoporous framework were implanted into certain organs in vivo, a great amount of active molecules or proteins in body fluid might be then captured and adsorbed, resulting in modified surface and improved bioactivity which might facilitate tissue responses. It has been reported that hydroxyapatite with microstructure leads to significantly higher albumin adsorption and has a positive influenced on osteoblast proliferation as a consequence, which indicates adsorbed proteins acts at least partly in regulating cell responses [33].

Having been shown critical for osteoblast adhesion and sequential functions during osteoblasts/materials interactions, bioactive apatite is an essential component of tooth and bone [34]. A bioactive material is considered to be the one able to form bone-like apatite selectively after it is immersed in a serum-like solution. The possible mechanism of apatite formation on silica xerogel is proposed: negatively charged silanol groups bond with calcium, phosphate, hydroxyls and so on, forming nuclei for apatite crystal growth. A high activation surface energy is required to form the nuclei in SBF [3]. MSXs with higher SSA were associated with lower surface silanol group density as demonstrated by TG analysis (Fig. 3). It was reported that

with the increase of SSA of MSX, the pore structures were transformed from disordered state to well-defined ordered MCM41 like structure [16]. And increasing in degree of long-range order (amorphous silica xerogel < MCM41 analogues < MCM48 < MCM41), the surface silanol groups decreased from 8–9 to ~3 μmol per m^2 [35]. Therefore, it is suggested that increasing SSA of MSX would result in higher long-range order, which consequently decreased surface silanol groups. In the following experiments, it was found that the apatite growth rate was significantly promoted on MSXs with higher SSA. It is assumed that MSXs with higher SSA have higher surface energy and show higher apatite formation capability in vitro. The mechanism of this phenomenon could be explained by Mkhonto and Leeuw computer stimulation study, in which they found that apatite does not attach strongly to hydroxyl groups on surfaces, on the contrary, apatite should deposit more readily at dehydrated silica surfaces, especially when the surface silicon and oxygen species rearrange to form O–Si–O links [36]. Therefore, our results support their computer simulation study in an experimental aspect. Greatly enhanced osteoblasts proliferation was observed after the formation of a hydroxycarbonate apatite layer [37]. Therefore, apatite layer formation should be responsible for the favorable osteoblastic proliferation on MSXs with higher SSA.

The deposited apatite layer and adsorbed serum proteins can change xerogel surface topography, chemistry and osteoblastic affinity. These subtle changes in local microenvironment can result in significant discrepancies in cell behaviors. The observed differences in osteoblast proliferation may be partially regulated by integrin subunits expression and consequent signaling pathway. Additional understanding of MSXs with high SSA and their influence on osteoblast functions as well as bone regeneration in vivo will be further investigated.

5 Conclusions

In summary, MSXs with different SSA were prepared via surfactant-template approach by varying ammonia/silica molar ratio. We demonstrate that osteoblastic proliferation was promoted by MSXs with higher SSA markedly via up-regulated integrin α5 at an early stage during cell attachment. It can be rationalized that the higher serum protein adsorptive capability and faster apatite layer formation rate in MSXs with higher SSA are associated with favorable osteoblast proliferation. Our results demonstrate MSXs with high SSA could serve as better controlled-release fillers, giving an alternative on drug-loading-releasing profiles, and also improving the tissue responses or even tissue engineering.

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