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Ultrafast Spreading Effect Induced Rapid Cell Trapping into Porous Scaffold with Superhydrophilic Surface

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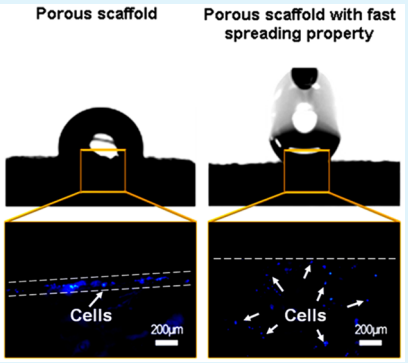
Supporting Information

ABSTRACT: In this contribution, superhydrophilic chitosan-based scaffolds with ultrafast spreading property were fabricated and used to improve the trapped efficiency of cells. The ultrafast spreading property allowed cells to be trapped into the internal 3D porous structures of the prepared scaffolds more quickly and effectively. Cell adhesion, growth, and proliferation were also improved, which could be attributed to the combination of UV irradiation and ultrafast spreading property. The construction of ultrafast spreading property on the scaffold surface will offer a novel way to design more effective scaffold in tissue engineering that could largely shorten the therapeutic time for patients.



KEYWORDS: *ultrafast spreading, cells trapping, scaffold, superhydrophilicity, tissue engineering*

The interaction between cells and materials is one of the most important essential issues in tissue engineering and regenerative medicine, which is strongly related to the regulation of cells behaviors on the materials, such as adhesion, growth, proliferation and differentiation.[1−7] The adhesion process is so complex, besides the influence of the biological behavior of the cells themselves, including cell metabolism, charges, flexibility, and hydrophobic/hydrophilic state on the cytomembrane,[8−10] and it is also affected by the chemical compositions, structures, charges and mechanical strength of the materials. Wettability, as an important integrated external expression of the physical and chemical properties of materials, is employed to investigate cells behaviors on the materials in recent years.[11−14] As reported, cells exhibited well-adhered ability on the surface with moderate wettability from hydrophobicity to hydrophilicity.[15,16] Lee et al. constructed gradient wetting surface by grafted different charged functional groups including acrylic acid, sodium p-styrenesulfonate and N,N-dimethyl aminopropylacrylamide on smooth polyethylene surface, on which the surface contact angle (CA) ranged from 30 to 90°.[15] They found that the optimized adhered behavior of cells was exhibited on the surface with CA ∼50°.

Besides the moderate wetting surfaces, cells adhesion on those with superwettabilities (superhydrophobicity ∼CA higher than 150° and superhydrophilicity ∼CA closed to 0°)[17,18] is attracting more and more interests due to the importance on regulating cells behavior and understanding the basic process of cells adhesion, growth and proliferation.[19−23] By using a phase-separated method, superhydrophobic poly(L-lactic acid) (PLLA) was prepared. The surface wettability could be controlled from superhydrophobic to superhydrophilic by Ar plasma irradiation.[7] The superhydrophobic PLLA surface inhibited the mouse fibroblast cells L929 adhesion in first 24h, and superhydrophilic PLLA surface showed good biocompatibility for cells than that on the superhydrophobic one. The optimized cells adhesion behavior was also found on the moderate wetting surface with CA ∼80°. Moreover the proliferation behaviors of cells adhered on the special wettable surfaces was investigated and the cells behaviors exhibited more complex. For example, fibroblast L929 cells showed good proliferation behaviors on the superhydrophobic polystyrene surface; on the contrary, osteoblast SaOs-2 cannot proliferate on it.[23] This proliferating inhibition of SaOs-2 on the superhydrophobic surface can be changed by UV irradiation. It was found that after UV irradiation, SaOs-2 could proliferate on the treated PS surface with hydrophilic and superhydrophilic properties. One could conclude that, compared to the superhydrophobic surface, cell adhesion can be performed more easily on the superhydrophilic surface. However, it can be seen that most cells adhesion researches on the superhydrophilic surfaces are mainly focused on the two-dimensional surfaces in a static condition, there are few studies on how the liquid spreading property of the superhydrophilic surface affect cell adhesion in three-dimensional structures of scaffolds. Moreover, the efficiency of cell trapping into the scaffold is crucial for tissue regeneration, which process is typical drove by

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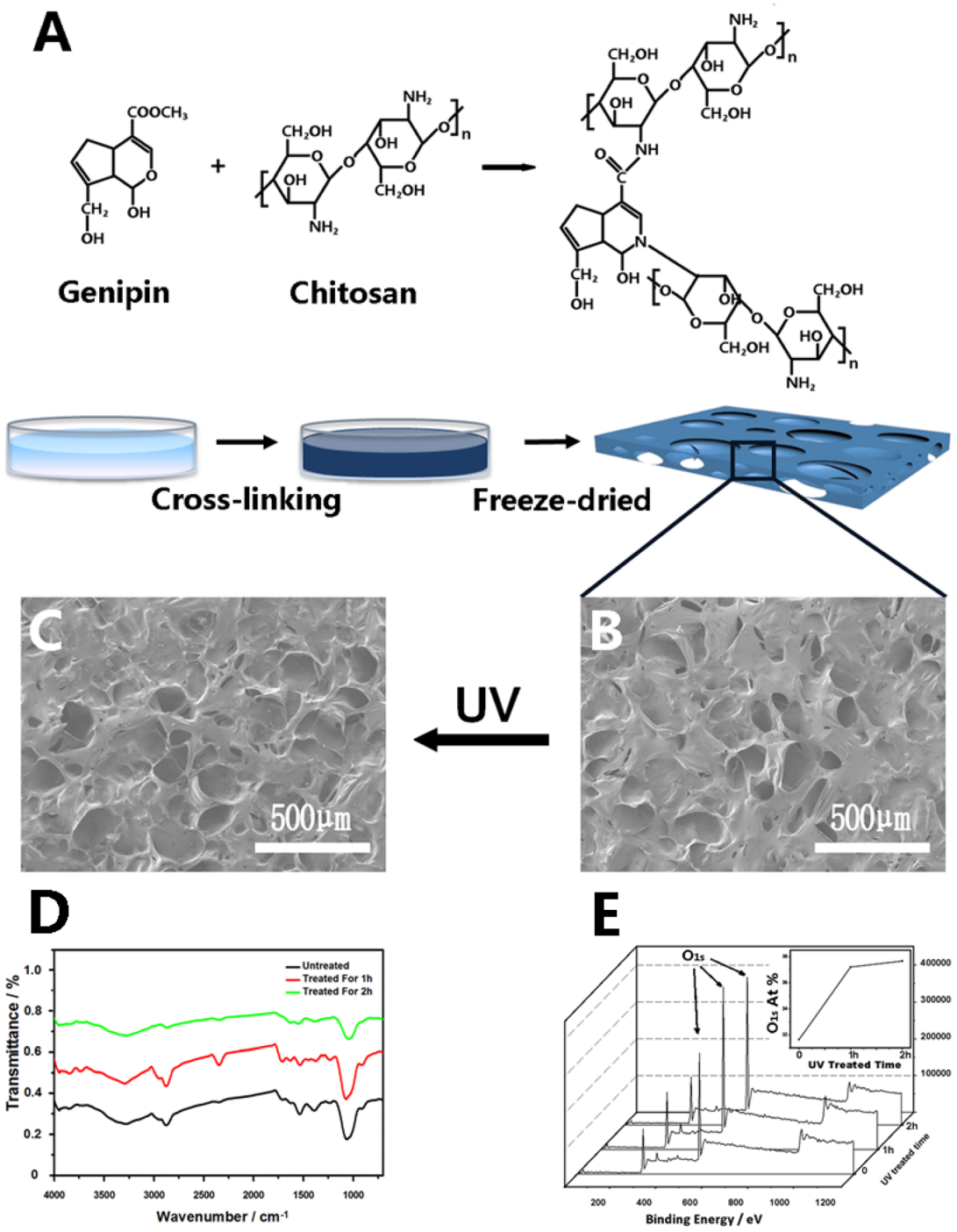

Figure 1. (A) Typical prepared process of chitosan scaffold; (B, C) SEM images of CG10 chitosan scaffold surface without treatment and treated by UV light for 2 h, respectively; (D) attenuated total reflectance Fourier transform infrared spectroscopy of the CG10 chitosan surface treated by UV light for 0, 1, and 2 h; (E) X-ray photoelectron spectroscopy of CG10 chitosan surface treated by UV light for 0, 1, and 2 h. It indicated that the hydrophilic groups (hydroxyl, carbonyl, and carboxyl groups) on the chitosan scaffold surface raised with the increasing of UV treated time from 31.64 to 37.66% showed on the top right corner.

the motility of cells themselves and takes much time in day-scale that would increase the practical therapeutic period.[24] Therefore, it is reasonable and necessary to look for a novel way to accelerate the trapping process of cells into the scaffold. Superhydrophilic surface with ultrafast spreading property offer an opportunity to allow the seeding cells suspension permeating into the porous structures quickly because of the drive of three-dimensional capillary forces.

In this contribution, proof of concept was demonstrated by the chitosan-based scaffold due to its wonderful properties of well biocompatibility, biodegradability, nontoxicity and easy preparation.[25−27] Superhydrophilic scaffold surface with ultrafast spreading property was achieved by UV irradiation. Further cell experiments showed that cells could be trapped into the porous structures of chitosan scaffold with ultrafast spreading property more easily and faster than the untreated one. Correspondingly the trapping process of cells into the scaffold

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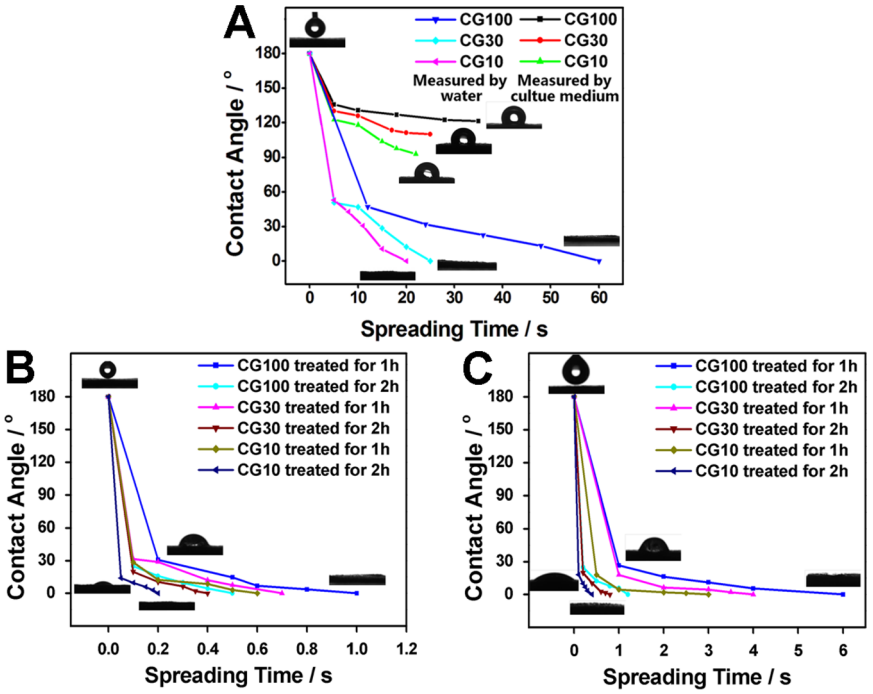

Figure 2. (A) Spreading behaviors of water and culture medium on the untreated CG10, CG30, and CG100 surface. It showed that water could spread on all prepared chitosan porous scaffold surface completely. However, the culture medium solution could not spread on the untreated CG10, CG30, and CG100 surface, and exhibited the final CAs of 92.9 ± 7.8, 109.8 ± 3.3, and 121.2 ± 1.4°, respectively; (B) water spreading behaviors on the CG10, CG30, and CG100 surface treated by UV light for 1 and 2 h; (C) culture medium spreading behaviors on the CG10, CG30, and CG100 surface treated by UV light for 1 and 2 h.

was greatly accelerated. Cells behaviors, such as adhesion, growth, and proliferation, were also improved on the prepared chitosan scaffold because of the cooperative effect of ultrafast spreading property and UV irradiation.

A typical prepared process of the porous chitosan scaffold is shown in Figure 1A. In brief, chitosan solution was first cross-linked by the natural cross-linker genipin with different doping rates of 10:1, 30:1, and 100:1, which were abbreviated as CG10, CG30, and CG100, correspondingly.[28] After being cross-linked, the prepared hydrogel was freeze-dried for 12 h, and then the porous chitosan scaffolds were obtained. During this process, redundant water in the hydrogel network was removed and the inner structures that remained resulted in the porous structures in the scaffold. With increasing doping amount of genipin in the chitosan solution, cross-linking degree of the porous chitosan scaffolds increased correspondingly, during which the color changed from light white to dark green as shown in Figure S1A−C. It could be attributed that the amount of double bonds in genipin molecules for cross-linking increased with the dosage of genipin.[29] SEM image in Figure 1B showed that the prepared CG10 scaffold surface was full of microscaled pores, the size of which was more than 100 μm. The profiled image of CG10 in Figure S2 showed that the chitosan scaffold was full of macropores (>100 μm), and small micropores were also found on the macroporal wall. From these structures analyses, the scaffold should be interconnected and could be used for the subsequent biomedical applications. The pore size on the CG100 and CG30 scaffolds shown in Figure S1E, F decreased slightly that might be attributed to the insufficient introduce of cross-linking agent. Porosities of the CG10, CG30, and CG100 shown in Table S1 are 96.1, 80.2, and 73.6%, correspondingly.

To achieve superhydrophilicity on the chitosan surface with ultrafast spreading property, the scaffolds were treated by UV light with a power of 4600 microwatts/cm^2 for 0, 1, and 2 h. As shown in Figure 1B and C, there was no obviously difference on the structures of CG10 surfaces before and after UV irradiation for 2 h. Regarding the CG30 and CG100, similar results could be also found in Figure S1, which demonstrated UV irradiation could not change the surface structures significantly. The attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectra in Figure 1D showed that there was no obvious difference between the surfaces of CG10 treated by UV light for different times, which meant that the surface chemical compositions had no essential changes after UV irradiation. However, after UV irradiation, the C−C bonding would occur homolysis. And then peroxyl radicals were generated in the presence of oxygen, which was the key intermediate of the subsequent thermal reactions leading to hydroxyl, carbonyl, and carboxyl groups. Among this process, the content of O_{1s} is usually employed to quantitative analyze the density of the hydrophilic groups. The X-ray photoelectron spectroscopy (XPS) in Figure 1E showed that the percentage of O_{1s} on the CG10 surface increased from 31.64 to 37.66% with the increase of irradiation time from 0h to 2h as shown on the top right corner of Figure 1E. It meant that the hydrophilic groups on the CG10 surface, such as hydroxyl, carbonyl, and carboxyl groups, increased with the irradiation time. It could conclude that the combined effect of the increasing of hydrophilic groups and the porous structures on the chitosan surface resulted in the unique ultrafast spreading property.

Because the culture medium was a mixture solution composed of inorganic salts, amino acids, serum, and so on, the relative viscosity of 1.1184 is higher than that of pure water, and its surface tension of 59 dynes is lower than that of water, ~72 dynes. It would lead to different wettable behavior

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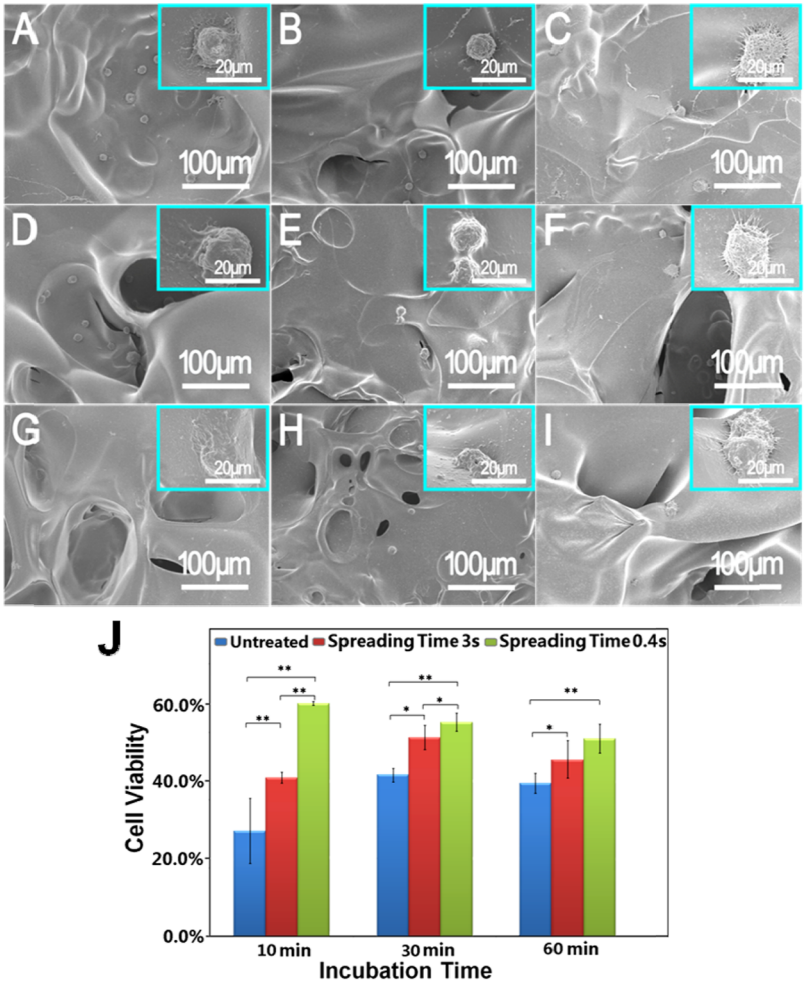

Figure 3. (A–C) SEM images of the untreated CG10 surfaces that were cultured by MC3T3 cells for 10, 30, and 60 min, correspondingly; (D–F) SEM images of the CG10 surfaces with CMST ~3.0 s that were cultured by MC3T3 cells for 10, 30, and 60 min, correspondingly; (G–I) SEM images of the CG10 surfaces with CMST ~0.4 s that were cultured by MC3T3 cells for 10, 30, and 60 min, correspondingly; (J) cell viability on CG10 with different CMST in culture for 10, 30, and 60 min blue column represented untreated chitosan sample; red column represented surface with 3.0 s spreading time; green column represented surface with 0.4 s spreading time. Statistically significant difference compared to another group *($P < 0.05$), **($P < 0.01$).

compared to water. Therefore, wettability of both water and culture medium were considered in the experiment. For the smooth chitosan surface, the CAs of both water and culture medium were higher than 90°, as shown in Table S2, which showed a weak hydrophobic property. There was no too much wettable difference between water and culture medium. However, the surface wettability would exhibit an obvious change with the introduction of structure and UV treatment. As shown in Figure 2A water CAs on all these untreated porous scaffolds of CG10, CG30 and CG100 were around 0° that was considered to be induced by the water permeation into the microporous chitosan scaffold. Nevertheless, the water spreading time (WST) of CG10, CG30, and CG100 were strongly affected by the doping amount of genipin, it increased with the decreasing doping amount, which was 20, 25, and 60 s, correspondingly shown by rose, watchet, and blue line in Figure 2A. The slower water spreading time for the untreated scaffold might be resulted of the small pore size. Meanwhile, among these three samples, the fastest spreading rate on CG10 might be attributed to the highest porosity among these three samples. Regarding the culture medium, it would spread slightly on the porous chitosan surface and then become stable; the culture medium spreading times (CMST) were 22, 25, and 38 s showed by the green, red, and black lines, respectively, in Figure 2A, which had similar tendency of water spreading behavior on these surfaces. The final CAs of culture medium on CG10, CG30, and CG100 were 92.9 ± 7.8, 109.8 ± 3.3, and 121.2 ± 1.4°. Besides the smaller pore size in the scaffold, the relative higher viscosity of culture medium might be another important reason for the unspreading property on the scaffold. After UV irradiation, both water and culture medium spreading times on these surfaces decreased dramatically, as shown in Figure 2B, C. Water spreading processes on these prepared scaffold surfaces were showed in Figure 2B. When the surface was irradiated by UV light for 1 h, the WSTs of CG10, CG30, and CG100 were 0.6, 0.7, and 1.0 s, respectively. When we further increased UV irradiation time to 2 h, the WSTs of CG10, CG30, and CG100 would decrease to 0.2, 0.4, and 0.5 s. It can be seen that the WST decreased with the increasing of genipin amount in the hydrogel for the same UV irradiation time; meanwhile, it also decreased with the UV-treatment time increasing. Regarding to the culture medium, the CAs on the prepared scaffold surfaces became 0° regardless of whether the UV irradiation time was 1 or 2 h, as shown in Figure 2C. The

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CMSTs of CG10, CG30, and CG100 after 1 h UV irradiation were 3.0, 4.0, and 6.0 s, respectively; when the UV irradiation time is increased to 2 h, the spreading rate of culture medium increased and corresponds to CMST of CG10, CG30, and CG100 decreased to 0.4, 0.8, and 1.2 s. On the basis of the above results, it can be seen that, compared to the water spreading behavior, the spreading tendency of culture medium was similar; namely, it decreased with the increase in genipin amount and UV treatment time; on the other hand, the spreading rate of culture medium was slight slower than that of water spreading behavior, which might be attributed to the higher relative viscosity of culture medium compared to that of water.

To investigate how the ultrafast spreading property affected cells trapping process into the scaffold and subsequently biological behaviors, we used the CG10 scaffold because of its fastest spreading property among the prepared scaffold samples; and murine osteoblastic MC3T3-E1 cells were employed as model cells in the biological assay. In Figure 3A, it can be seen that there were several cells on the untreated chitosan scaffold surface that exhibited spherical conformation after 10 min culture; the cells adhered on the surface via filopodia gradually with increasing the culture time of 30 min (Figure 3B) and 60 min (Figure 3C). After UV irradiation for 1 h, the CMST on the chitosan scaffold surface became 3.0 s. Compared to the cells on the untreated scaffold, MC3T3-E1 cells expressed relative spreading conformation, as shown in Figure 3D–F, corresponding to the culture time of 10, 30, and 60 min. When further increasing the UV irradiation to 2 h, the CMST became 0.4 s. The cells exhibited relative flat conformation on the surface after 10, 30, and 60 min culture, shown in Figure 3G–I, correspondingly; and there were few cells observed on it. On one hand, the oxidative and hydrophilized chitosan surface induced by UV irradiation would facilitate the adsorption of cell adhesion-mediating proteins and the serum of the culture media, which were favorable for binding of specific amino acid sequences to integrin receptors of the cells.[12,23,30] Cell adhesion was improved and the cell conformation expressed more and more flat with the UV irradiation time increase. On the other hand, because of the hydrophobicity of culture medium on the untreated scaffold surface, the cells could not be trapped into the scaffold by the permeating effect but stayed on the surface; the ultrafast spreading property allowed cell suspension to enter the inner part of the prepared scaffolds quickly.

MTS assay in each short-term culture time of 10, 30, and 60 min was also used to identify this effect as shown in Figure 3J. The cell viability rose with the decreasing spreading time in each culture time, which meant that the faster the spreading property on the scaffold surface, the more cells in the scaffold. It could be attributed to the cell trapping efficiency on the scaffold with ultrafast spreading property being higher than that on the untreated one. Additionally, one could not find the statistical difference between each culture time; it might be a result of the insufficient cell proliferation in such a short time. Usually, this improvement in cell adhesion on the scaffold was considered to be the contribution of UV treatment; however, the surface observation by SEM and fluorescent microscopy in Figures 3G and 4B showed there were few cells found on the treated scaffold surface and almost all the cells were inside of scaffold because of the fast spreading property induced by the strong 3D capillary force, as shown in Figure 4D. Therefore, it could conclude that the ultrafast spreading property on the

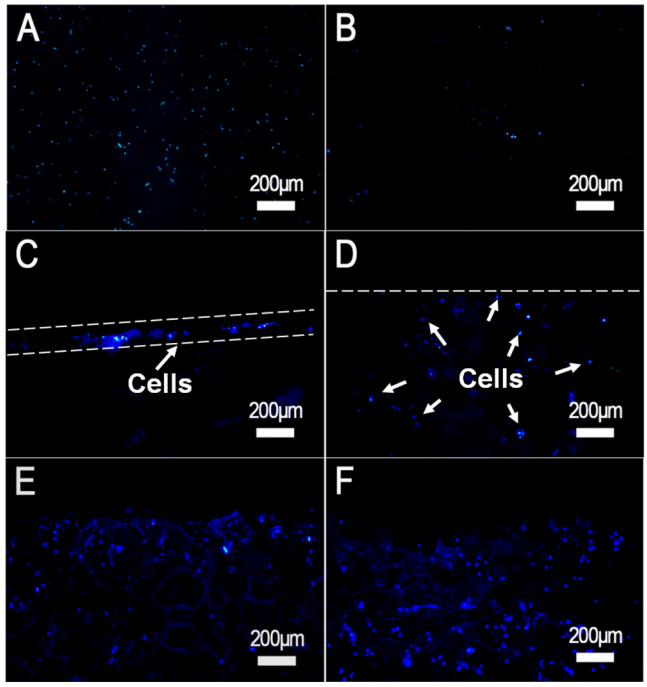

Figure 4. (A, B) Fluorescent images of cells adhesion on the untreated CG10 scaffold and the superhydrophilic CG10 scaffold surface with ultrafast spreading property cultured for 10 min in top view; (C, D) corresponding fluorescent images of cells adhesion in profiled view; (E, F) fluorescent images in profile view after 1 day culture. MC3T3 cells were stained by DIPI.

scaffold surface gave the main contribution on the improvement of cell trapping. With increasing the culture time for 1, 3, and 7 days, SEM images in Figure S3 showed that cells adopted more flat conformation on the CG10 with ultrafast spreading surface compared to those on the untreated one or that with CMST of 3.0 s, correspondingly. An MTS assay in Figure S3J also showed that cell viability on the scaffold with ultrafast spreading property was higher than that on the untreated one or that with a 3.0 s spreading time. It demonstrated that there were more MC3T3 cells adhered on the scaffold with ultrafast spreading property than others.

Following investigation of fluorescent microscope in Figure 4 would be further used to analyze cell behavior on the prepared scaffolds. From the top view, there were many cells adhered on the untreated CG10 surface corresponding to the DAPI-stained blue dots after 10 min culture, as shown in Figure 4A; there were few cells adhered on the treated one with ultrafast spreading property, as shown in Figure 4B. From the profile view, one could find that the cells were full of the inner treated CG10 scaffold in Figure 4D; on the contrary, there were few cells observed inside the untreated scaffold, but a light blue line in Figure 4C was found on the surface, which meant most cells were still adhered on the surface. Even after 1 day culture, the amount of cells migrated into the untreated scaffold was significantly fewer than that into the treated one as shown in Figure 4E, F. This result was consistent with the MTS results.

In conclusion, porous chitosan scaffold was fabricated by a simple freeze-dried method. UV treatment with different times was employed to achieve ultrafast spreading property on the scaffold surface. An appropriate time of UV-exposure was necessary for the enhancement of hydrophilicity and water spreading rate. The biological results illustrated that the

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ultrafast spreading property allowed cells to be trapped into the internal 3D structures of the prepared chitosan scaffold more quickly and effectively; and cells adhesion, growth and proliferation were also improved combined with the UV irradiation. The construction of ultrafast spreading property on the porous scaffold surface will offer a novel way to design more effective scaffold in tissue engineering and regenerative medicine that could largely shorten the therapeutical time for patients. The forthcoming work might be focused on enlarge the variety of scaffolds with this ultrafast spreading and permeating properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b04009.

Experimental section; porosity of the prepared scaffolds; CAs of water and culture medium; optical photos and SEM images of the prepared porous chitosan scaffolds; bioanalysis of the cells culture experiments (PDF)

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Author Contributions

†C.W. and C.Q. contributed equally. C.W. prepared the samples, analyzed physical and chemical properties, and prepared the manuscript; C.Q. performed all the cell culture experiments and related bioanalysis; W.S. supervised, revised the manuscript ,and set the scientific aims; H.S. supervised and set the scientific aims.

Notes

The authors declare no competing financial interest.

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