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Nanoscale hybrid coating enables multi-functional tissue scaffold for potential multi-modal therapeutic applications

Zhijun Guo^{1,3†}, Nan Jiang^{2†}, Jessica Moore⁴, Colin P. McCoy⁴, Monika Ziminska⁴, Cormac Rafferty⁵, Gianluca Sarri⁵, Andrew R. Hamilton⁶, Yubao Li³, Li Zhang³, Songsong Zhu^{2*}, Dan Sun^{1*}

1. School of Mechanical & Aerospace Engineering, Queens University Belfast, Belfast, BT9 5AH, UK

2. State Key Laboratory of Oral Diseases, & National Clinical Research Center for Oral Disease, & West China Hospital of Stomatology, Sichuan University, Chengdu, China

3. Analytical and Testing Center, Sichuan University, Chengdu 610064, China

4. School of Pharmacy, Queens University Belfast, Belfast, BT9 7BL, UK

5. School of Mathematics and Physics, Queens University Belfast, Belfast, BT7 1NN

6. Faculty Engineering and the Environment University of Southampton, SO17 1BJ, UK

*Corresponding authors: d.sun@qub.ac.uk; zss_1977@163.com

† First and second author contributed equally

Abstract

Through a nature-inspired layer-by-layer assembly process, we developed a unique multi-functional tissue scaffold that consists of porous polyurethane substrate and nanoscale chitosan/ graphene oxide hybrid coating. Alternative layers of drug laden chitosan and graphene oxide nanosheets were held together through strong electrostatic interaction, giving rise to a robust multilayer architecture with control over structural element orientation and chemical composition at nanoscale. Combined pH-controlled co-delivery of multiple therapeutic agents and photothermal therapy have achieved by our scaffold system. The new

platform technology can be generalized to produce other tissue scaffold systems and may enable potential multi-modal therapeutic applications such as bone cancer managements.

Keywords: hybrid coating, tissue scaffold, multi-modal therapy, co-drug delivery, photothermal therapy

1. Introduction

Multi-modal therapeutics are highly desirable under certain challenging clinical settings such as management of bone cancers (such as osteosarcoma and bone metastases). Traditional treatment of bone cancers involves surgical removal of cancerous tissue, the use of radiotherapy / chemotherapy (cancer drugs) to manage pain and control tumor growth / recurrence.¹ Other treatments may also be required for combatting surgery-induced infections and promoting functional recovery. Bone scaffold that can fill the large bone defects while offering therapeutic functions such as controlled drug release for symptom (anti-tumoral, pain) relief, infection control, preventing cancer recurrence and promoting bone regeneration, are promising treatment protocol for reducing the risk of systemic effects and improve patient's quality of life.

To date, research on multi-functional bone scaffolds that offer multi-modal therapeutics is still in its infancy. Wong et al.² and Palamà et al.,³ developed drug-laden polycaprolactone (PCL) scaffolds for the postsurgical care of osteosarcoma. The scaffold porosity was achieved by press-molding or solvent casting / particulate leaching technique; however it is difficult to control the pore size/interconnection and porosity of the scaffolds. Zhang et al.,⁴ printed three dimensional (3D) PCL composite scaffold containing mesoporous bioactive glass and Fe₃O₄ nanoparticles (NPs). The magnetic scaffolds have uniform and continuous pore structure with improved mechanical properties (compressive strength and toughness) and can offer sustained cancer drug release. However, harsh/hazardous solvents are necessary for the preparation of the printable materials, which may present challenges on the drug bioactivity and *in vivo* toxicity of the scaffold. In addition, despite the biocompatibility of magnetic Fe₃O₄ NPs, they could be toxic to cells when concentrations are more than 0.05 mg /L.⁴ In recent years, photothermal therapy (PTT) which employs heat generated from absorbed near-infrared (NIR)

light to ablate cancer, has developed rapidly in tumor treatment. Various photothermal agents, including gold nanoparticles⁵ and other emerging nanostructures such as MoS₂ nanosheets⁶ and CuFeSe₂⁷ nanocrystals have been incorporated into bone scaffolds to enable photothermal therapy in bone cancer treatment. A recent study also reported a 3D printed bifunctional graphene oxide (GO)-modified β -tricalcium phosphate composite scaffold with combined photothermal effect and significantly improved bone-forming ability.⁸ However, to the best of our knowledge, there has been no 3D bone scaffold available to date to provide combined PPT and drug delivery, especially co-delivery of multi-therapeutic agents to meet the demanding requirements in bone cancer management.

In this study, we designed and fabricated a unique scaffold system which consists of porous polyurethane (PU) foam substrate with drug laden graphene oxide (GO) nanosheet /chitosan (CS) hybrid coatings. PU can be produced with a wide range of mechanical and physical properties, depending on its composition and synthesis procedure.^{9,10} Researchers in the past have successfully modified the biomechanical properties of PU to fit specific tissue engineering applications.^{11,12} In this study, biocompatible and biodegradable polyurethane (PU) with open porosity >70% was used as the model template in this study as it mimics the trabecular bone structures^{13–15}. GO nanosheets have high specific surface area with abundant negatively charged surface functions and has been used widely for the loading / delivery of various therapeutic agents^{16,17} and sensing of biological species.¹⁸ Being an electroactive material, GO can also achieve electrically-induced drug-release⁸ and cell growth.¹⁹ On the other hand, GO is one of the most promising candidates for photothermal cancer therapy due to its strong NIR absorbance, high-photothermal-conversion efficiency, excellent thermal conductivity, and strong potential in stimulating bone regeneration.¹⁹ Biocompatible and degradable CS was chosen as the polycation polymer and its abundant surface functional groups (hydroxyl and amino) and positive surface charge can enable the loading of various therapeutic agents (e.g, anti-cancer drugs,²⁰ growth factors,²¹ anti-microbial agents,²² *etc.*) and interact electrostatically with GO to form a robust coating. We hypothesize that the proposed hybrid coating system will have the combined advantage from its constituent materials and will provide several important functionalities to the tissue scaffold, including delivery of multiple

therapeutic payloads, providing on-demand local photo-thermal therapy and offering excellent biocompatibility.

2. Results and discussion

2.1 Materials characterization

Scheme 1. Layer-by-layer assembly of CS/GO hybrid coating on porous PU scaffold

Here we deployed a nature-inspired layer-by-layer assembly (LBL) process to produce hierarchical multilayered materials that mimic the structure of nacre and bones.²³ Alternative layers of oppositely charged organic/inorganic building blocks were deposited onto an organic substrate forming a multi-layer hybrid coating, see **Scheme 1** (more detailed description see Experimental Methods). Alternative layers of CS and GO were held together through a strong electrostatic interaction, giving rise to a multilayer architecture with control over structural element orientation and chemical composition at nanoscale. As both GO and CS can be loaded with model drugs such as fluorescein sodium (FL), methyl blue (MB) or Ag nanoparticles (AgNPs), different coating systems such as (GO-CS)_n, (n denotes number of bilayers), single or dual drug loaded (GO/FL-CS)_n, (GO-CS/MB)_n, (GO/FL-CS/MB)_n and (GO-CS/AgNPs)_n, have been produced following the similar LBL process. Theoretically, the coating thickness and choice of drugs for the hybrid coating can be customized according to the patient's specific disease conditions. The optical image in **Figure 1 (a)** shows that the scaffold color turns darker with increasing number of CS/GO bilayers, indicating a greater amount of GO has been successfully deposited. The SEM insets suggest that the coating process has no effect on the

high level of interconnectivity of the porous scaffold. **Figure 1 (b)** shows the cross-sectional SEM image of a typical coated foam strut and **Figure 1 (c)** is the close-up image showing the morphology of a 30 bi-layer hybrid coating. The thickness of a 5-bilayer coating is $\sim 0.65 \pm 0.32 \mu\text{m}$ and $\sim 3.90 \pm 1.37 \mu\text{m}$ for a 30-bilayer coating, according to SEM observation of different samples. The coating thickness was also confirmed by AFM (**Figure 1 (d)**). The result is consistent with the SEM analysis, where the average thickness for each bi-layer is $\sim 130 \pm 20 \text{ nm}$.

Figure 1. (a) Optical images of PU scaffolds with different number of bilayer coatings; inset: SEM image showing the scaffold microstructures before (top left) and after (top right) the coating deposition (b) cross-sectional SEM image of a coated foam strut (c) high magnification SEM image showing coating with 30 bilayers, (d) AFM image of 3 bilayers deposited on a Si substrate.

Figure 2. (a) FTIR spectra, (b) Mean compressive elastic modulus of scaffold (average taken from 5 scaffolds for each type of scaffold) with different number of bilayers (c) TGA, inset: magnification of the 5% weight loss, heat rate 10 °C min⁻¹ and (d) DTG spectra of PU scaffold with and without hybrid coatings.

Figure 2 (a) shows the FTIR spectra of the scaffold with different coating thicknesses. The gradually decreased PU characteristic peak intensity indicates the increased coating thickness as the LBL assembly process continues (**Figure 2a**). Compressive mechanical testing within the elastic range of the scaffold samples was conducted and an increasing trend in modulus was observed as the number of coated bilayers increased from 5 to 20, see **Figure 2 (b)**. The compressive modulus for samples with 20 bilayer coating (0.123 ± 0.0122 MPa) was double that of the control PU samples (0.053 ± 0.0091 MPa) without affecting the interconnectivity of PU scaffold (See **Figure 1 (a)** SEM insets). While the compressive modulus of the PU foam and that of the subsequent coated scaffolds are not comparable to natural bones, the main focus of this study is to establish a platform technology for fabrication

of multifunctional coatings. The technique can actually be generalized and applied to a wider range of surfaces (such as Si) and different scaffold substrates (such as mechanically stronger 3D printed PU scaffold, see **Figure S1** in Supporting Information). The thermal and thermo-oxidative stability of the scaffolds were studied by thermogravimetric analysis (TGA) in a nitrogen atmosphere. **Figure 2 (c)** and **Figure 2 (d)** shows the TGA and DTG spectra of scaffolds with and without coatings. Pure CS exhibits about 7.5% weight loss in the temperature range of 50-140 °C due to the evaporation of the absorbed water. The major weight loss of 57.9% between 250-400 °C is due to the degradation of polysaccharide units, which is in accordance with previous reports.^{18,24–28} The TGA curve of pristine PU foam demonstrated two main thermal events²⁹ : 1) 28.4% weight loss around 210-330°C, which is related to liberation of di-isocyanates due to depolymerization of the urethane and the disubstituted urea groups, 2) 97.63% weight loss around 340-600 °C, which can be associated to the decomposition of the remaining polyether chain. These two thermal events are consistent with the two peaks in Figure 3(d). From Figure 3(c) inset, it can be seen that the temperature at 5% weight loss ($T_{5\%}$) increased slightly with increasing number of bilayers when compared to that of the pure PU, and peak $T_{5\%}$ in DTG and peak at $T_{380^{\circ}\text{C}}$ (**Figure 2 (d)**) red-shifted indicating improved coating thermal stability with increasing coating thickness. The coated samples presented almost similar weight loss but higher coating thermal stability for the temperature range 200-500 °C. Comparing to pristine PU samples (residue 2.3622%), more solid residue (2.847%) remained for coated-PU which can be ascribed to the CS residual. The improved coating thermal stability may be a result of the excellent thermal stability of GO,²⁵ and the strong interaction between CS and GO which restricts the polymer motion on PU surface during the heating.³⁰

To investigate the electrical properties of the hybrid coating, Si wafer was deployed as a model substrate to facilitate measurements. The electrical resistance of the hybrid coating was measured under ambient conditions using an Agilent 34450A5½ Digital Multimeter following a standard four terminal set-up.³¹ The electrical conductivity of (GO-CS)₁₀ was 0.056 S/m, which is comparable to other CS/carbon system^[41–42] reported in the literature and is within the conductivity range of natural tissues such as ventricular muscle, blood, skeletal muscle

(0.03-0.6 S/m),^{25,32,33} and brain,³⁴ see Table 1. Such feature may enable the application of our coated scaffold for electrically induced sensing,³⁵ drug release,²⁴ and tissue regeneration,^{18,36,37} etc.

Table 1. Comparison of electrical conductivity with materials reported in the literature

Samples	Conductivity (S/m)
CS/carbon (dry) ²⁵	0.25 ± 0.09
CS/carbon (hydrated) ²⁵	0.04 ± 0.02
Ventricular muscle ³³	0.03
Body ²⁵	0.2
Blood ²⁵	0.6
Brain ³⁴	0.12
Skeletal muscle ³²	0.125
CS(dry) ³⁸	$7.4 \times 10^{-09} \pm 1.0 \times 10^{-09}$
CS (hydrated) ³⁹	0.03±0.02
This study: (GO-CS) ₁₀	0.056

2.2 Functional characterization

2.2.1 Single drug release from GO

Model drug fluorescein sodium (FL) can be loaded onto GO through formation of hydrogen bonding.⁴⁰ The overall zeta potential of GO/FL is -39.5 mV as is shown in **Figure 3(a)**. The molecular weight of FL is 376 g mol⁻¹, similar to several important drugs such as the cancer drug cisplatin, the non-steroidal anti-inflammatory indomethacin, as well as various bone regeneration growth factors (FTY20, Sphingosine-1 phosphate, Vitamin D3, etc).⁴¹ When dissolved in neutral aqueous medium, FL gives a distinct absorption peak between 450 to 500 nm which can be detected by UV-vis spectroscopy (see **Figure 3(b)**). **Figure 3(c)** shows an initial burst release followed by stable release of FL (~ 65%) in the neutral aqueous environment over the first ~12 h of immersion. From 12 h to 240 h the cumulative release

profile remains stable (~65%). In contrast, there was no FL release detected under pH 4 for up to 240 h. GO is hydrophilic and soluble under pH 7.4. Under pH 4, it becomes less hydrophilic and tends to aggregate, and hence may impede the drug release.^{38,42} On the other hand, GO has much stronger negatively charged surface under neutral condition due to the ionization of the hydroxyl and carboxyl groups,⁴³ see **Figure S2**. The greater negative surface charge density on the GO under pH 7.4 may repel the FL as there are two negative charges spread over four O atoms in its structure.⁴⁴

Figure 3. (a) Zeta potential of as-prepared GO, FL and GO/FL, (b) UV-vis spectra of as-prepared GO, FL and GO/FL, (c) cumulative release (%) of FL from (GO/FL-CS)₂₀ PU scaffold under neutral (pH 7.4) and acidic (pH 4) conditions, (d) zeta potential of as-prepared CS, MB and CS/MB, (e) UV-vis spectra of as-prepared CS, MB and CS/MB, (f) cumulative release (%) of MB from (GO-CS/MB)₅ scaffold under neutral (pH 7.4) and acidic (pH 4) environment.

2.2.2 Single drug release from CS

Methylene blue (MB) was selected as a model drug to investigate the release profile from the scaffold containing drug-loaded CS. MB is negatively charged and has a molecular weight similar to that of many low-molecular weight drugs and growth factors used for bone regeneration.⁴⁵ **Figure 3 (d)** shows the zeta potential of as-prepared MB, CS, and MB loaded CS (CS/MB). Through electrostatic interaction, MB was successfully loaded onto CS, giving CS/MB an overall zeta potential of 53.9 mV. **Figure 3 (e)** shows the UV-vis spectra of as-prepared CS, MB and CS/MB, respectively. The spectra of CS/MB overlaps with that of the MB, showing strong characteristic peaks at 668 nm and 292 nm, which indicates the successful loading of MB. The effect of pH on the MB release profile has also been investigated for (GO-CS/MB)₅ samples immersed in acidic (pH 4) and neutral (pH 7.4) aqueous solutions, respectively. From **Figure 3 (f)**, it can be seen that the release of MB under both pH conditions undergoes a burst release at the initial stage, after which the drug concentration stabilized at averages of around 55% under pH 4 and 30% under pH 7. It has previously been reported that the swelling of CS plays an important role in the drug release process, and the release is mainly controlled by diffusion⁴⁶. The pH dependent MB release can be attributed to the surface protonation of CS surface amino groups under pH 4. This would lead to polymer swelling which enhances the network porosity and facilitates the drug release through diffusion⁴⁷. At neutral pH, the amino groups are uncharged and the CS molecular chains return to a more compact mode and the diffusion process is suppressed.⁴⁸ Such pH responsive drug release profile is of particular interest for localized cancer treatment. On-demand release of anti-cancer drug can be triggered by the acidic tumor microenvironment, and the exposure to such drugs

can be minimized under healthy conditions (neutral pH) to reduce the associated side effects.

2.2.3 Dual drug release from CS/GO hybrid coating

While some researchers have demonstrated the feasibility of anti-cancer drug delivery from tissue scaffolds,⁴⁹ scaffold systems offering controlled release of multiple therapeutic agents (e.g., pain relief, cancer drugs, antimicrobial agents, growth factors, etc) are scarce. The complex interplay between the materials, therapeutic payloads and the surrounding physiological environment present major challenges in the creation of a highly functional system. Nevertheless, such systems are highly desirable for applications such as the management of bone cancers, as multiple therapeutic agents, if supplied in an appropriate fashion, may offer higher treatment efficacy and promote tissue regeneration simultaneously. To investigate the feasibility of co-delivering multiple drugs from our scaffold, FL loaded GO and MB loaded CS were deposited onto the scaffold using the same LBL process resulting in (GO/FL-CS-MB)₂₀ samples. Results (**Figure 4**) show that dual release of FL and MB can be achieved by the hybrid coating, with the pH dependent release profile of FL being similar to the results shown in 2.2.1. MB release has been identified under both pH conditions, however, it is hard to distinguish the pH dependence of the MB release from the dual drug system from the UV-vis spectra. This may be because the MB characteristic peak has been shifted due to the interference of the strong signal from GO/FL across the whole wavelength range under neutral conditions (also see **Figure 3(b)**).

Figure 4. Drug release from (GO/FL-CS/MB)₂₀ scaffold in (a) pH 4 and in (b) pH 7.4 during the first hour, cumulative release (%) of (c) MB and (d) FL for 240 hours.

2.2.4 Anti-microbial properties

Patients undergoing bone tumor surgeries are at high risk of postoperative deep infection caused by bacteria. Such complications present significant health challenges and usually require additional surgery, prolonged use of antibiotics, and delays of other scheduled treatments.⁵¹ To address this issue, we incorporated silver nanoparticles (AgNPs) into the CS/GO hybrid coating to serve as antimicrobial agents for a broad spectrum of Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains.^{52,53} In addition, AgNPs have also demonstrated anti-cancer properties against various types of cancer cells such as human hepatoma cells⁵⁴ lung cancer⁵⁵ breast cancer⁵⁶ and cervical carcinoma,⁵⁷ *etc.* The overall zeta potential of AgNPs loaded CS (CS/AgNPs) is ~ 56.4 mv, see **Figure 5 (a)**. The overall positive charge of the CS/AgNPs indicates it can still form a hybrid coating with the negatively charged GO through the previously described LBL process. Negatively charged AgNPs were loaded onto CS as described in the experimental section. The UV-vis spectra of CS/AgNPs overlaps with that of the AgNPs and the strong characteristic peaks at 398nm

indicates the successful loading of AgNPs (**Figure 5 (b)**). *S.aureus* (ATCC 6538) and *E.coli* (ATCC 11303) were used for antimicrobial evaluation. **Figure 5 (c)** shows that the AgNPs loaded scaffold displayed anti-bacterial ability against both *S. aureus* and *E.coli*. The number of bacteria drastically decreased ($68.85\% \pm 2.87$ of *S.aureus* and $98.75 \pm 0.73\%$ of *E.coli*) in the inoculum containing the AgNPs loaded scaffold (Figure 7(d)), confirming its effectiveness in antimicrobial applications.

Figure 5. (a) zeta potential of as-prepared CS, AgNPs and CS/AgNPs; (b) UV-vis spectra of as-prepared CS, AgNPs and CS/AgNPs; (c) Viable *S.aureus* (CFU/mL) and *E.coli* remaining in the wells containing control PU and (GO-CS/AgNPs)₂₀ after 4 hours of incubation; (d) reduction of bacteria relative to the control PU.

2.2.5 Photothermal property

It is known that the GO exhibits strong light absorption ability in the near-infrared (700-900 nm) region,⁵⁸ and can effectively convert light energy into heat due to the delocalization of the electron states.⁵⁸⁻⁶⁰ **Figure 6** shows the IR thermal images of PU control and (GO-CS)₂₀

with hybrid coatings under CO₂ laser (808 nm, 0.15 mW) irradiation for up to 10 min. The uncoated PU scaffold showed no temperature rise throughout the test period whereas the average temperature of the coated scaffold (at the point of irradiation) increased from 25°C to 45 °C within 30s. The average temperature on (GO-CS)₂₀ scaffolds remained stable both in air and PBS solution throughout the rest of the test. It is possible to adjust the photothermal conversion efficiency of the GO containing scaffold through manipulating the GO concentration, the NIR power density and/or the size of the scaffold.³⁸ The temperature of the scaffold under irradiation can be adjusted to 40-44 °C, i.e., within the therapeutic window for cancer hyperthermia.^{61,62} With the NIR being able to penetrate tissues with sufficient intensity and high spatial precision, it is expected that our scaffold can be tailored to offer localized photothermal therapy when required. Additionally, photothermal effect has also been shown to facilitate anti-cancer drug release from graphene based nanocarriers,⁶³ hence our hybrid coating modified scaffold could also serve as an ideal platform offering multi-modal therapy for bone cancer treatment with the option of stimuli induced drug release.

Figure 6. IR thermal images of (a) PU control and (GO-CS)₂₀ in (b) air and (c) PBS solution under 808 nm laser irradiation.

2.2.6 *In vitro* cell behavior

Cell morphology

SEM images in **Figure 7A** shows the degree of BMSCs adhesion on our scaffolds after 2h incubation. The cells on the control group (PU scaffold), show more globular morphology with limited spreading despite abundant filopodia were found on the cell leading edge. In contrast, BMSCs on (GO-CS)₂₀ scaffold samples exhibited much better spreading featuring triangular, ellipsoidal or polygonal morphologies. Apparently, cells attached to the (GO-CS)₂₀ scaffold demonstrated more visible inter-connected lamellipodia, and the number of BMSCs on (GO-CS)₂₀ scaffolds are much higher than PU scaffolds, which suggests that (GO-CS)₂₀ favors better cell viability and stronger cell adherence.

The more clearly organized cytoskeletons were shown in the CLSM images in **Figure 7B**, which further confirms the BMSCs adhesion behavior on our scaffolds. Vinculin is a protein significantly involved in cell adhesion dynamics and, in particular, focal adhesion maturation, whereas cell adhesion is one of the most significant steps for cellular functions such as survival and differentiation.⁶⁴ It is proposed integrin β 1 protein can mediate cell adhesion and integrate signals from a variety of cytokines and other growth factors. NELL-1 (potent osteo-inductive factor) binding to integrin β 1 activates an intracellular signaling cascade that promotes cell adhesion, proliferation, and osteogenic differentiation.⁶⁵ It can be seen that the expression of both integrin β 1 and vinculin manifested more abundant expression (much stronger fluorescent intensity) on the (GO-CS)₂₀ scaffold in comparison to the pure PU scaffold after 4 h of culturing.

The much stronger fluorescent intensity of integrin β 1 and vinculin expression on (GO-CS)₂₀ scaffold suggest the coated scaffold shows stronger cell adhesion than the PU control sample.^{65,66}

Figure 7. A: Initial cell adhesion after incubation for 2h, (a) PU scaffold, (c) (GO-CS)₂₀ scaffold, B: The expression of integrinβ1 and vinculin detected by immunofluorescence assay, and the merged images of integrinβ1 (green), vinculin (red) and DAPI nuclei on scaffold. Bar=10μm.

The proliferation of cells was qualitatively determined by CCK-8 assay, see **Figure 8**. All

groups exhibit gradually increased OD values from day 1 to day 5, suggesting a continuous cell proliferation in each group. It can be seen that both PU scaffold and (GO-CS)₂₀ scaffold can induce cell proliferation over a 5-day period, further confirming our multi-functional scaffolds exhibit excellent biocompatibility and can be used for potential *in vivo* applications.

Figure 8. CCK-8 assay proliferation after 1, 3 and 5 days of culture, n=5 per group; (*), $p < 0.05$ when compared with blank group, (#), $p < 0.05$ when compared with PU control group.

Conclusion

In this study, we have created bio-compatible porous tissue scaffolds with a unique nanoscale hybrid coating consisting of alternating layers of graphene oxide nanosheets and chitosan, through a nature-inspired layer-by-layer assembly process. The coating is electrically conductive and can impart improved thermal stability and mechanical properties to the PU substrate. Results show that our coated scaffold can offer pH-dependent delivery of multiple therapeutic agents, as well as anti-microbial properties and localized photothermal therapy. The structure / functionality of the scaffold can be tailored through nanoscale control over the coating architectures according to the specific needs of the diseases. We envisage our method can be generalized to create functional surfaces for a wide range of hard/soft scaffold materials

(e.g., metals, ceramics, polymers, 3D printed structures, composites, etc) and the coating can be loaded with other therapeutic agents such as practical cancer drugs and growth factors. With modern healthcare technology paving its way towards customized solutions for best patient outcomes, our multi-functional scaffold can potentially revolutionize the way for future multi-modal therapeutics in the biomedical field.

3. Experimental Methods

3.1 Fabrication of the multi-layer CS/GO hybrid coating

Open-cell polyurethane (PU) foam with 30 pores per inch (PPI) was supplied by the Foam Shop Ltd, UK. Graphene oxide (GO, 2mg/mL, dispersion in H₂O) and Chitosan (CS, low molecular weight 50,000-190,000 Da, 75-85% deacetylated) were purchased from Sigma Aldrich. The PU foam was cut into cylindrical foam templates (12.7 mm in diameter and 10 mm in height) for further coating deposition. As illustrated by Scheme 1, PU foam templates were first immersed in 1M NaOH solution for 10 min, thoroughly rinsed with DI water, and dried in oven at 37 °C for 12 hours prior to coating deposition. The foam templates were then immersed in polyethylenimine (PEI, Sigma Aldrich branched, average Mw ~25000, 0.01 M) solution for 60 min to generate a primer layer followed by rinsing with DI water. The LBL coating was formed by immersing the foam templates into GO (2mg/ml) and 0.5% wt CS solution (in 2% (v/v) acetic acid aqueous solution) alternatively. The immersion time was 30 min in each solution, and the samples were rinsed and dried between immersions. This procedure was repeated until a desired number of layers was achieved. The as-prepared specimens were denoted as (GO-CS)_n, where n indicates the number of GO/CS bilayers formed on the PU templates.

3.2 Loading of model drugs

Fluorescein sodium (FL, 1.2 wt%, Sigma Aldrich) and methylene blue (MB, Sigma Aldrich) were used as model drugs for the preparation of drug release test. GO nanosheets were loaded with FL by adding 1.2 wt% FL to 2 mg/mL GO aqueous solution, stirring overnight at

room temperature, then sonicating for 48 h. Unbound FL was removed through centrifugation (3500 rpm for 6 h). The FL loaded GO (GO/FL) was then re-dispersed in DI water by sonication for 12h. The final FL loading on GO is 32.4% according to TGA analysis. MB loaded CS (CS/MB) was obtained by adding MB into prepared CS solution (0.2 mg/mL) and stirred under 37°C for 8 h. Following similar LBL assembly technique shown in Scheme. 1, three types of drug loaded samples were prepared, namely (GO/FL-CS)_n, (GO-CS/MB)_n and (GO/FL-CS/MB)_n, where n denotes the number of GO/CS bi-layers. Silver nanoparticles (AgNPs, 10nm diameter, Sigma Aldrich) were added into the CS solution (0.02mg/mL) and sonicated for 4h, and used to prepare (GO-CS/Ag)_n samples.

3.3 Characterization

The surface charge of GO and CS were determined by zeta potential analysis using a Malvern Zetasizer Nano instrument with a 633 nm laser. All data were averaged over three measurements. Transmission FTIR was conducted under ambient conditions using a Perkin Elmer spectrometer. Resolution was set to 4 cm⁻¹. Thirty-six scans per sample were recorded, averaged and corrected against the spectrum of attenuated total reflection crystals as background. Thermogravimetric analysis (TGA, TA Instruments SDT-Q600) was carried out over a temperature range 40 to 600 °C (heating rate 10 °C min⁻¹) under a nitrogen atmosphere. The weight of all samples was kept within 9-13 mg in an open alumina pan. Scanning electron microscopy (SEM) images were taken with a JEOL JSM-7001 F4 microscope, using acceleration voltage in the range of 2-15kV. Prior to SEM, samples were cryo-fractured in liquid nitrogen and sputter coated with gold. To better confirm the LBL coating thickness and its electrical conductivity, GO/CS hybrid coating has also been deposited onto clean Si substrate following the same LBL technique as described earlier. The thickness of deposited coating was then measuring using atomic force microscopy (AFM, Cypher ES system, Santa Barbara, USA) under tapping mode with AC160TS cantilevers. The electrical conductivity of the coating was measured using an Agilent 34450A5_{1/2} digital multimeter following methods reported elsewhere^{39,67}.

The release of model drugs was characterized using UV-Vis spectroscopy (Cary Win UV)

under pH 7.4 and pH 4, respectively. The relative characteristic peak intensities (450 nm for FL, 665nm for MB) were compared at different time interval to determine the drug release profile as a function of time. The photothermal effects of specimens were tested under 808nm CO₂ laser irradiation (0.15 W cm⁻²) in an ambient environment. The temperature of the specimen was monitored by an FLIR i60 thermal imaging camera, with images frequency of 9 Hz and IR resolution of 180 x 180 pixels. The thermal images were further analyzed by FLIR R&D software. Quasi-static mechanical testing in compression was conducted using a Lloyd's LRX Tensile Tester with 50N load cell following ASTM D1621-101 with non-standard specimen sizes (12.7 mm diameter, 10 mm height). Specimens were deformed at a displacement rate of 0.5 mm s⁻¹, within the linear elastic range (approximately 0.6 mm displacement). The elastic modulus of each samples was calculated as the slope of the stress-strain plot in the linear portion of the curve.

3.4 Antibacterial testing

Escherichia coli (E. coli) ATCC 11303 and *Staphylococcus aureus* (S. aureus) ATCC 6538 were chosen as standard bacteria and were incubated in Mueller-Hinton Broth medium. PU control and (GO-CS/AgNPs)₅ samples were sterilized and placed in 24-well cultures. The bacteria were seeded on these samples with a concentration of 1×10^6 CFU/mL plate in an incubator at 37°C for 4 h. After 4 hours, 200 µL of inoculum within the wells was removed by pipette, serially diluted and spread on Mueller-Hinton agar plates and incubated at 37 °C overnight for the colonies count.

3.5 In vitro studies

3.5.1 Isolation and culture of bone marrow stem cells

Cell isolation and culture were conducted following the standards established by the Animal Research Committee of the State Key Laboratory of Oral Diseases and West China School of Stomatology, Sichuan University (WCHSIRB-D-2018-003). Briefly, bone marrow stem cells (BMSCs) were harvested and extracted from the femurs of 4-week-old male Sprague-Dawley rats (Animal Research Center, Sichuan University, China) and cells at passage 2-4 were used for the cell experiment in this study.

3.5.1 Cell morphology

After 2 hours of cell incubation (5×10^4), the scaffold samples were rinsed three times with copious amount of PBS to remove unattached cells. The remaining cells were fixed with 2.5% glutaraldehyde (Sigma-Aldrich). The cell morphology was analyzed using SEM. More specifically, the cells were dehydrated in a series of ethanol solutions (20%, 40%, 60%, 80%, 90%, and 100%) and then gold sputtered for SEM analysis.

3.5.2 Cell immunofluorescence staining

Cell immunofluorescence staining, the expressions of integrin $\beta 1$, vinculin, on the experimental samples were detected by CLSM. After incubation with the samples for 4h, the cells were fixed in 4% paraformaldehyde, and permeabilized with 0.2% Triton X-100. Subsequently, 4', 6-diamidino-2-phenylindole (DAPI, company, country) and specific antibodies targeting protein of interest were added sequentially and co-incubated for 4 h as previously described. All procedure was completed in dark and all samples were observed using CLSM after thorough rinsing using PBS.

3.5.3 Cell proliferation

L929 cells (CCL-1) derived from normal subcutaneous areolar and adipose tissue of C3H/An mouse were obtained from American Type Culture Collection (ATCC) and the cells were used for general biocompatibility tests according to ISO10993. Cells were first cultured in low glucose DMEM (Dulbecco's modified Eagle's medium, Gibco Laboratories, Gaithersburg, USA) supplemented with 5% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (Gibco Laboratories, Gaithersburg, MD, USA). Then the cells were incubated at 37 °C in a 5% CO₂ humidified atmosphere. The proliferation rate of L929 cells was estimated using a cell counting kit (CKK-8; DOJINDO Laboratories, Japan) assay.

3.6 Statistical analyses

Data were expressed as mean $\pm$ standard deviation (SD) and analyzed by using the SPSS 16.0 software (SPSS Inc., USA). Significant differences among the groups were assessed using the one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) multiple comparison test. Statistical significance was defined as $p < 0.05$.

Associated content

Supporting Information

The Supporting Information is available free of charge on the XXX website at XXX.

Author information

Corresponding Authors: Dan Sun, Songsong Zhu

Tel: +44 (0) 28 90974701

E-mail: d.sun@qub.ac.uk; zss_1977@163.com

Author Contributions

† N. Jiang and Z. Guo contributed equally to this work.

Z. Guo and D. Sun conceived ideas of this work. Z. Guo carried out all experimental work in relation to materials synthesis and characterization. N. Jiang performed all biology related work. Jessica Moore carried out all bacterial related work. C. Rafferty and G. Sarri contributed to the laser related tests. All authors contributed to the analysis of data and result discussion as well as the writing of the manuscript.

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