Response to Reviewer 3 Comments

The manuscript reviews extensively the use of Chitosan-based scaffolds for endogenous bone regeneration. While the topic is very narrow, the authors have tried reviewing the field comprehensively. Few major suggestions:

Point 1: Extensive editing of the language required.

Response 1: We appreciate the reviewer for the advice. The amendments including grammatic mistakes, sentence errors and language organization in whole text have been checked and corrected thoroughly by a native English-speaking colleague. The related amendments have been highlighted in red color in revised manuscript.

Point 2: Some of the figures are not very professional. Figure 1- the arrow doesn't have any significance scientifically, looks more like an artwork; Figure 2- resolution very poor, FEBTE is not very clear and the building has nothing to do with the figure; Figure 6 - poor resolution.

Response 2: Thanks for your comments.
In fact, the arrow has great scientific significance in Figure 1 in the combination of the bow and arrow. The arrow represents the chitosan-based scaffolds proposed in this review, and the bow represents the keypoints for the design of multifunctional chitosan-based scaffolds. Visually, the open bow is used as the force-storing component of the launching arrow, which means that this review intends to functionally design the chitosan-based scaffold through three parts including the loading of osteoinductive molecules, hybridization with bioactive nanomaterials and physical stimulation. The arrow's direction implies chitosan-based scaffolds are intended to be used for facilitate endogenous bone regeneration. Therefore, by using the combination of bow and arrow, the main content and direction of this review are vividly explained, so that readers can quickly capture the content and purpose of this review.
Building FEBTE in Figure 2 is actually in analogy with buliding the world-famous Great Wall of China. The Great Wall of China, as an ancient Chinese military fortification, played an important role in defending against foreign invasion, which made use of the geographical advantages and exhibited the intelligence of Chinese. Similarly, FEBTE took adtantages of the intrisic biological environment of organism and played the predominant role in the field of bone repair. Although this strategy is simple, it is more convenient and practical in bone repair than the tedious BTE strategy.
In addition, the picture resolution of Figure 2 and Figure 6 has been improved in the revised manuscript as follows.

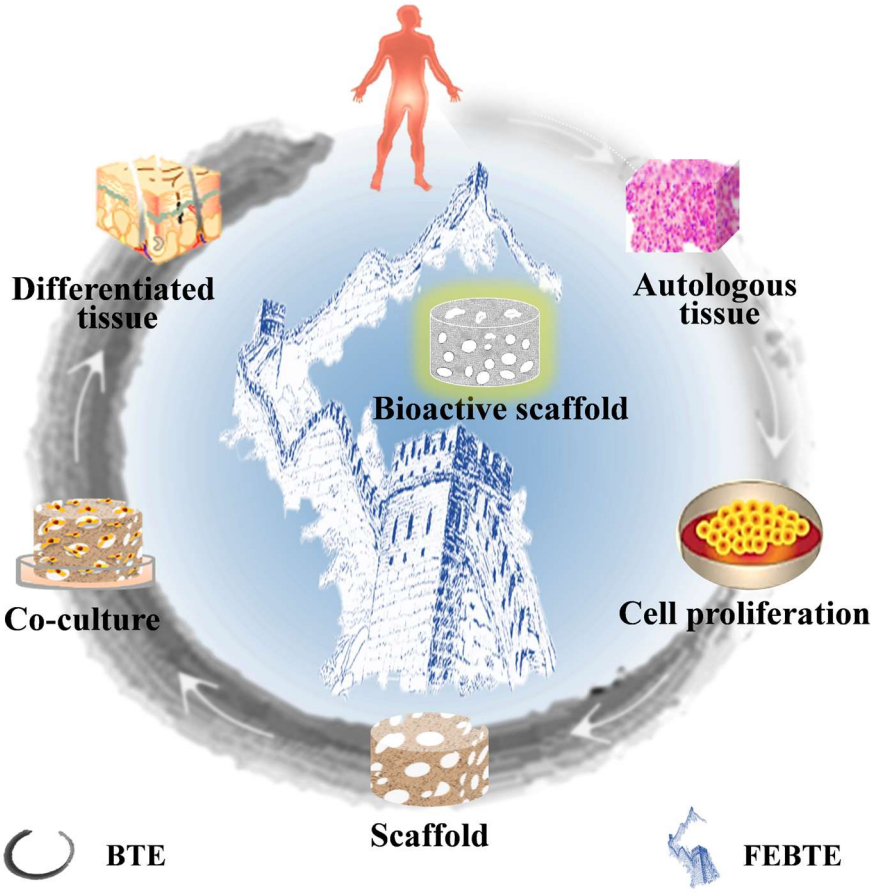

Figure 2. Comparison of two bone repair strategies. Traditional bone tissue engineer-ing (BTE) needs tissue harvest, cell isolation and co-culture with scaffold ex vivo, while facilitated endogenous bone tissue engineering (FEBTE) avoids these tedious and risky procedures by using a bioactive scaffold.

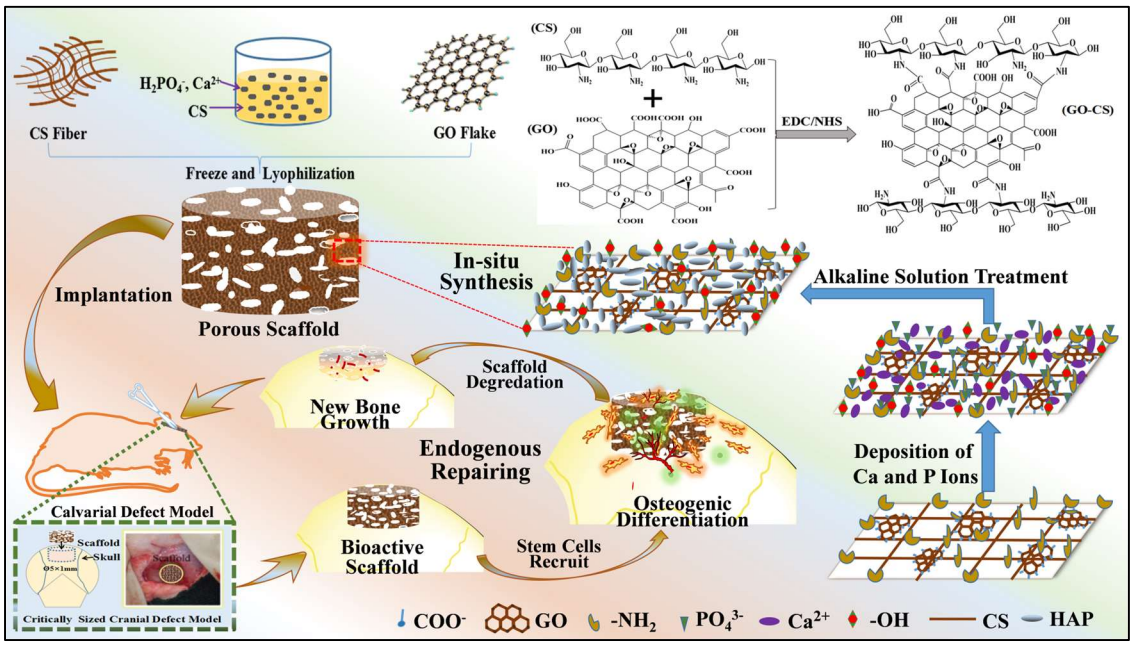

Figure 6. The GO/CS/nHAP scaffold prepared by in-situ mineralization strategy is used for endogenous bone regeneration[15].

Point 3: Section 3.2.3 on 3D Printing only talks about the general steps involved and the 3D printing techniques. A few paragraphs about the works on 3D printed CS-based scaffolds will add value to the manuscript.

Response 3: According to the comments of the reviewer, we have made the necessary supplementary carefully in revised manuscript as follows.

The first paragraph of section 3.2.3 of the text: "For example, Zafeiris et al. printed a CS/HAP scaffold at low temperature by controlling flow, infill and perimeter speed, which created a cell-friendly living environment and finally promoted cell adhesion and proliferation on its surface (*doi:10.1016/j.msec.2020.111639*)."

The third paragraph of section 3.2.3 of the text: "In recent years, fortunately, Olhero's team developed a series of sintered-free CS-based biphasic CaP bone scaffolds by robocasting suppressing sintering as post printing process (*doi.org/10.1016/j.msec.2018.09.050*). The absence of sintering enables the addition of biomolecules or functional nanoparticles to the extrudable inks, such as antibiotic levofloxacin and magnetic iron-doped HAP nanoparticles (*doi.org/10.1088/1748-605X/abac4c*). This enhanced fabrication technology endowed CS-based scaffolds with superior functions for cancer therapy by strong magnetic hyperthermia or infection treatment by bioactive drugs in some bone diseases. To enhance the strength of scaffold, the latest work presented by Torres et al. used this low temperature additive manufacturing technique to get a CS-based hybrid scaffold. By adjusting the ratio of silk fibroin in CS/CaP complex, the printing scaffold with macropore size 300 μm showed 17 MPa compressive strength and 0.26 GPa Young's modulus in dry state (*doi.org/10.1016/j.msec.2022.112690*)."

Point 4: A comprehensive table on CS-based scaffolds fabricated using different techniques and their properties can be added.

Response 4: Thanks very much for your valuable advice. We've added a comprehensive table on CS-based scaffolds fabricated using different techniques and their properties in revised manuscript as follows.

Table 1. The fabrication techniques and properties of CS-based scaffolds.

Fabrication techniques	Composite	Important properties	Ref.
Freeze drying	CS/graphene oxide/tetracycline hydrochloride	Controlled the drug release and promoted faster bone growth in rat femur defects	[77]
	CS/graphene oxide	Oriented pores enhanced the alignment of MC3T3-E1 cells, facilitated osteogenesis	[78]
Electrospinning	Zein/CS/Polyurethane /carbon nanotubes	Facilitated cell proliferation, differentiation and upregulated the expression of osteogenic proteins	[79]

	CS/HAP	Supported cell adhesion and promoted bone regeneration by activating integrin-BMP/Smad signaling pathway	[80]
	CS/poly (vinyl alcohol)/carbonated hydroxyapatite	Promoted cell adhesion, growth and osteogenesis	[81]
	CS/silk fibroin/cellulose	Osteo-immunomodulatory effects, accelerated bone regeneration in rat calvaria defects	[60]
3D printing	Silk fibroin/CS/CaP	Enhanced the strength of scaffold, facilitated the proliferation and osteogenic differentiation	[82]
	CS/HAP	Created a cell-friendly living environment, promoted cell adhesion, proliferation and osteogenesis	[83]
	CS/polyvinyl alcohol/SiO_2	Excellent mechanical properties and osteogenic differentiation ability	[84]
Sol-gel method	CS/bioactive glass	Good shape memory properties and geometrical accommodation in bone implantation	[85]
Gas foaming + microwave irradiation	CS/HAP	Scaffold with interconnective pores facilitated cells growth and upregulated osteogenic genes (RUNX2, OCN, COL I, ALP) expression	[86]
Freeze drying + porogen-leaching out	CS/HAP	Scaffold with gradient pore and HAP composition implemented the bidirectional repair of osteochondral defects	[87]

Point 5: Since the manuscript focuses on osteoinductivity, a table of osteoinductive CS-based scaffolds (drug loaded, protein, exosomes, etc that are discussed in section 4.1) and their results will really benefit the readers.

Response 5: We appreciate the reviewer for the comments. According to the suggestions of the reviewer, we've added a comprehensive table in section 4.1 of the revised manuscript for better understanding as follows.

Table 2. The osteoinductive CS-based scaffolds integrate with different molecules.

Types	Molecules	Composite matrix	Key results	Ref.
Drugs	Icariin	Carboxymethyl CS/HAP /poly(lactide-co-glycolide)	Improved the adhesion, proliferation and differentiation of MC3T3-E1 and finally achieved the repair of bone defects	[124]

	Ursolic acid	MHAP/CS	Upregulated the expression of osteogenic-related genes though promoting the M2-type polarization of macrophages	[123]
	Chrysin	CS/carboxymethyl Cellulose/HAP	Stimulated cell proliferation and promoted osteoblast differentiation	[38]
	VEGF, BMP-4	Gelatin/CS	Induced bone regeneration by angiogenesis and osteogenesis	[126]
Proteins	BMP-2	PCL/carboxymethyl chitosan	Supported the proliferation, differentiation and ossification of hBMSCs	[149]
	BMP-2, insulin-like growth factor-1	CS/gelatin	Significantly enhanced osteoblastic differentiation	[150]
Peptides	FRHRNRKGY (HVP), GRGDSPK (RGD)	CS	Significantly enhanced osteoblastic differentiation	[42]
	Parathyroid hormone-derived peptide	CS/HAP	Increased osteoblast adhesion, proliferation differentiation and calcium deposition	[71]
	Pulp stem cell-derived exosomes (DPSC-Exo)	CS	Remarkably stimulated new bone formation in rabbit radial defects (size: 1.5 cm)	[140]
Exosomes	human umbilical cord mesenchymal stem cells-derived exosome	HAP/silk fibroin/glycol CS/polyethylene glycol	Greatly facilitated the repair of alveolar bone and treated the periodontitis	[141]
	hMSCs-derived exosome	CS	Effectively recruited stem cells, promoted their proliferation and osteogenic differentiation, and finally mediated bone repair	[139]
	microRNA (siFlt-1+siCkip-1)	CS	Enhanced the osteogenesis and angiogenesis, finally promoted new bone regeneration in vivo	[147]
Genes	miR-24	CS/gelatin	Promoted osteogenic differentiation and skull defect regeneration in vivo	[151]
	miR-590-5p	CS/HAP /nano-ZrO_2	Upregulated osteogenic genes (RUNX2, COL I, ALP) expression and promoted osteoblast differentiation	[152]