Supplementary Information

Latent progenitor cell-stimulating therapy for regeneration of chronic tympanic membrane perforations using IGFBP2-releasing chitosan patch scaffolds

Materials and Methods

Effects of IGFBP2 on TM cells

Primary TM cells were obtained from TMs of 5 day-old SD rats. For *in vitro* testing, TM cells were cultured and maintained at 37°C in DMEM supplemented with 10% FBS in a humidified atmosphere with 5 % CO_2 for 2 weeks. *In vitro* studies using TM cells were conducted to determine the optimum concentration of IGFBP for incorporation into CPSs. TM cells were seeded on 24-well plates at 5×10^4 cells/well. Next, 20, 200 ng/ml and 2 μg/ml of IGFBP were added to the wells. After 1, 3, and 7 days, cell viability was measured using the water-soluble tetrazolium salt (WST-1) assay by adding WST solution to each well, and incubating the plates at 37°C and 5 % CO_2 for 4 h. The samples were then gently agitated for 1 h. Absorbance was measured at 540 nm using a microplate reader (Versamax Reader; Molecular Devices, Sunnyvale, CA, USA) to determine the optimal IGFBP concentration for use in the study.

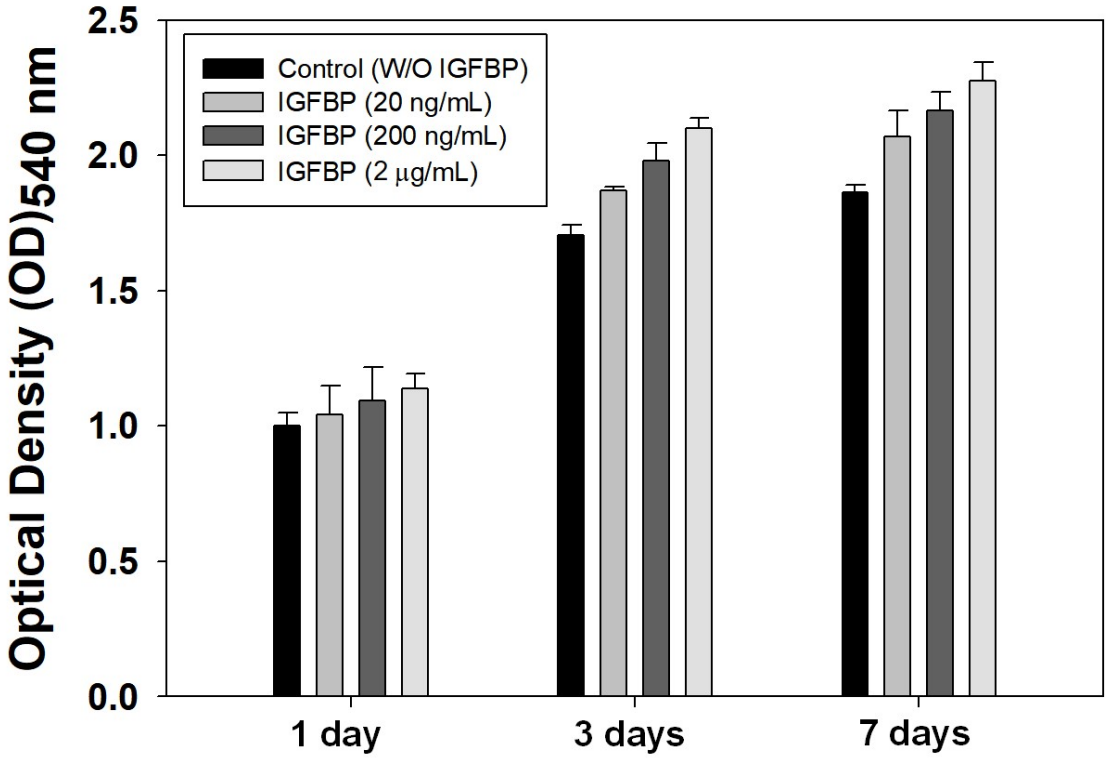
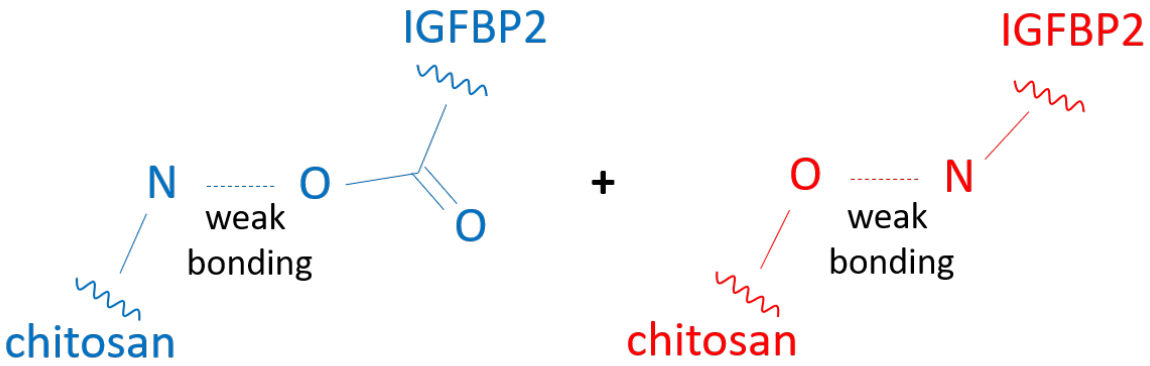


Figure S1. The water-soluble tetrazolium salt assay of tympanic membrane (TM) cells according to the concentration of insulin-like growth factor-binding protein (IGFBP) using *in vitro* cultures.



Mechanical strength ↑

Figure S2. Possible mechanism. Amine groups of chitosan and carboxylic groups of insulin-like growth factor-binding protein 2 (IGFBP2) can be involved in hydrogen bonding. In the same manner, hydroxyl groups of chitosan and amine groups of IGFBP2 can be involved in hydrogen bonding. These types of bonds can occur in many sites of patches, resulting in the reinforcement of mechanical strength.

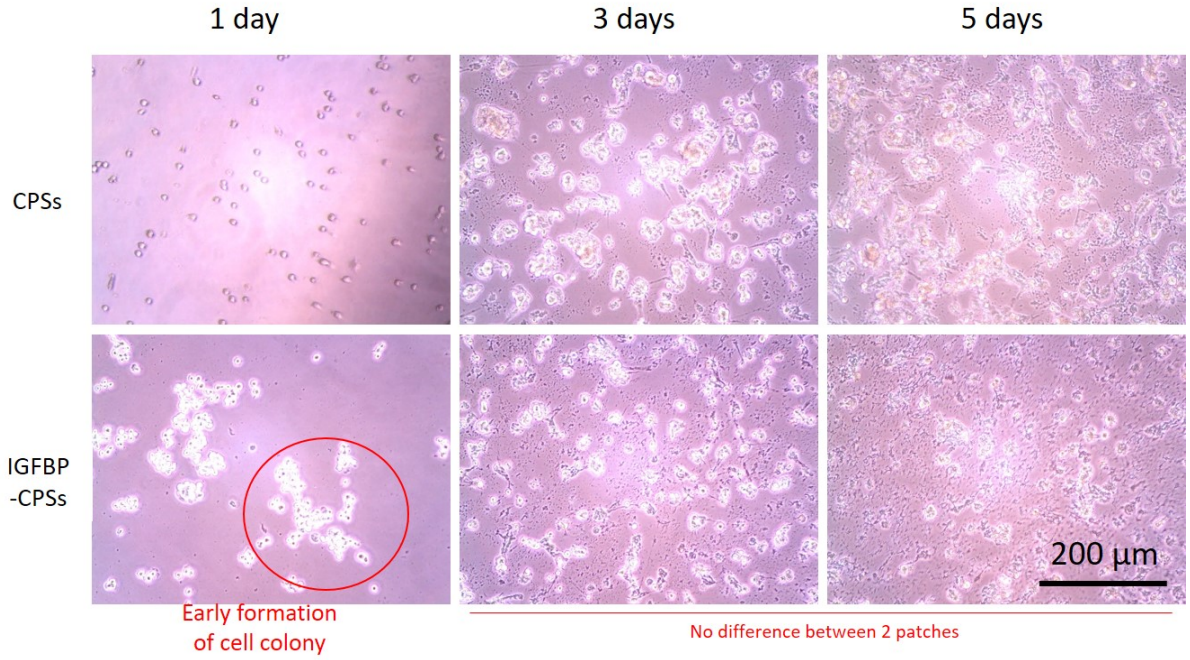
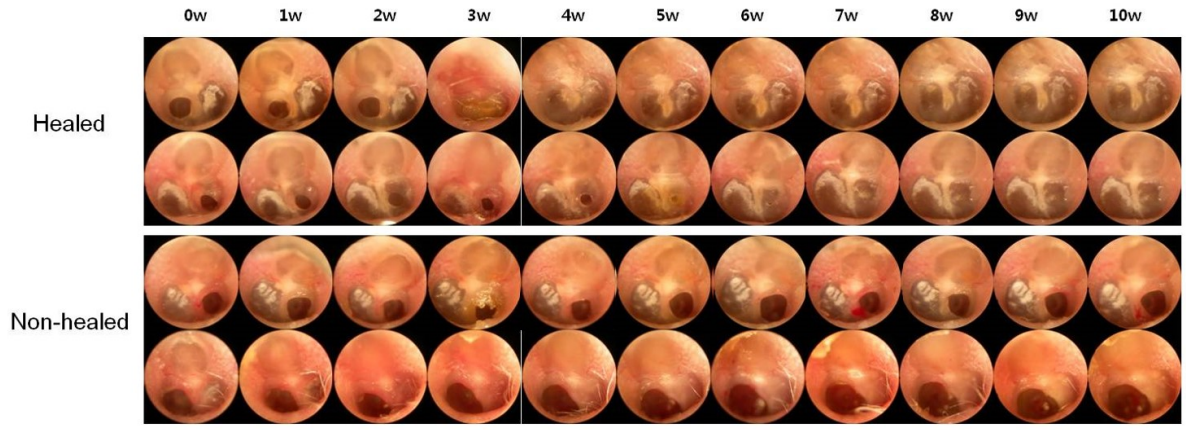


Figure S3. Representative images of TM cells grown on chitosan patch scaffolds (CPSs) and IGFBP-CPSs (×100). TM cells of the IGFBP-CPSs group aggregated with one other, presumably because of the effects of IGFBP2.



Week	1	2	3	4	5	6	7	8	9	10
16 ears	2	3	3	6	6	6	7	7	7	7/16 43.8%

Figure S4. Serial images and table showing the efficacy of IGFBP2-CPSs for chronic TM perforations.

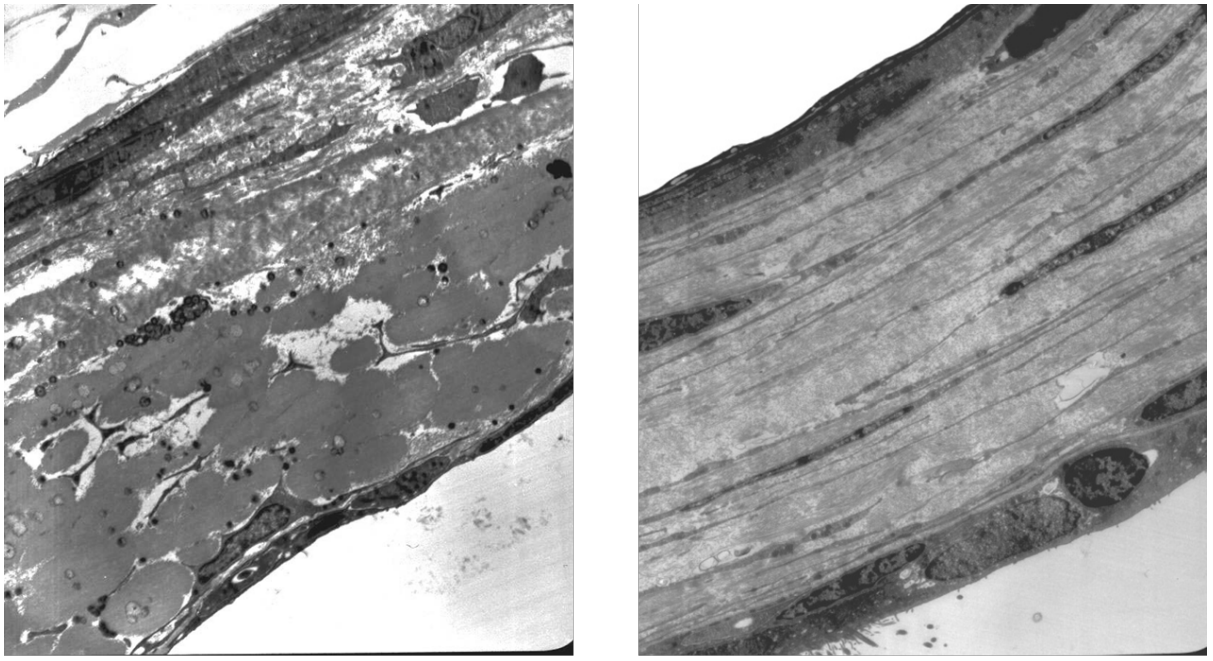


Figure S5. Representative images of TM cells grown on CPSs and IGFBP-CPSs (×100). TM cells of the IGFBP-CPSs group aggregated with one other, presumably because of the effects of IGFBP2.

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see:

http://www.textcheck.com/certificate/BIzdbN

(1) We recommend that you include the above statement in the end of your document to inform reviewers that the English has been professionally checked. If you do not, then you may receive the comment "English needs revision". Some reviewers who are not native speakers of English seem to add the comment "English needs revision" to EVERY paper they review by non-US authors... (The above text can be deleted later).

(2) We strongly recommend that you do not make ANY changes to this document. Textcheck should be the last step before final formatting. No further editing should be necessary unless there is text that the editors have misunderstood.

If any text has been misunderstood:
Rewrite the sentence(s) that you need to change, SAVE, and upload the completed document to:
http://www.textcheck.com/client/submit
for a final check. (Please do not mark your changes in any way and do not send the document to us by e-mail unless you have a problem with the www site.)
Requests for a final check should be made within 1 month.

[If you make changes to this document and require a re-edit (such as changes post review) then it should be uploaded via 'Submit Document' on our www site, with a note that the file is a revision of '19020810'. The fee for a revised document is based on the wordcount of changed and new sentences (only). As there is no charge for sentences that have not changed, we recommend that clients always upload complete documents.]