3D-printed polyurethane-based scaffolds for bone regeneration: a pilot study

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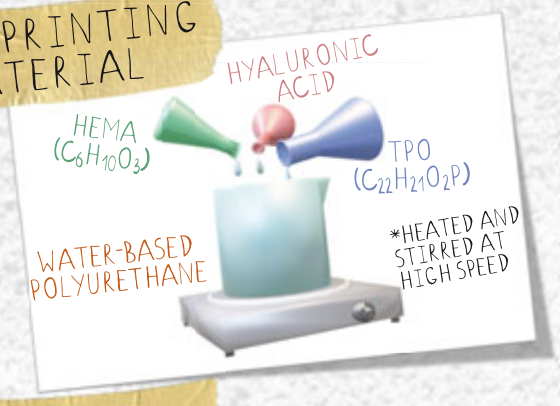
OBJECTIVE

The objective of this pilot study was to evaluate the viability and cellular migration of preosteoblasts (MC3T3-E1) on scaffolds made with a 3D-printable polyurethane-based material using a Miicraft 125 3D-printer (Miicraft®, Taiwan). In addition, the potential use of a platelet extract (PE) gel as a carrier of growth factors was evaluated to enhance cellular proliferation at imbibed scaffolds.

MATERIAL&METHODS

3D-PRINTING MATERIAL



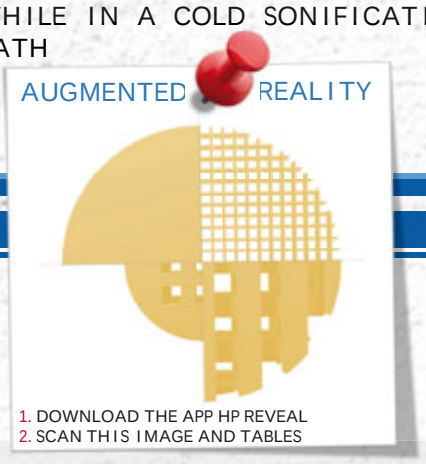
MIGRATION

3D-PRINTED SCAFFOLDS IMBIBED OR NON-IMBIBED EITH PE-GELWERE PLACED UNDER AN 8uM-PORE CELL-CULTURE INSERT WHERE MC3T3-E1 WERE SEEDED. CELL COUNTING WAS PERFORMED AFTER 24, 48 AND 72H USING CCK-8® (DOJINDO, JAPAN).

PE-GEL

1. CENTRIFUGATION OF HUMAN WHOLE BLOOD (BIOIVT, USA) AT 1,000G FOR 2′ 15″ AND A SECOND CENTRIFUGATION OF THE SUPERNATANT AT 1,000G FOR 5′.
2. THE PELLET WAS ISOLATED AND RESUSPENDED IN PLATELET POOR PLASMA.
3. THE SUSPENSION WAS LEFT ON COLD BATH SONIFICATION FOR 5′ AND THEN 1/10 VOLUME OF 10X PBS WAS ADDED.
4. 5- FOLD DILUTION WITH VITROGEL™ 3D (THEWELL BIOSCIENCE INC., USA).
5. SCAFFOLDS WERE IMBIBED ON PE-GEL WHILE IN A COLD SONIFICATION BATH

AUGMENTED REALITY



CELLULAR VIABILITY

EVALUATED WITH MC3T3-E1 AFTER DIFFERENT SCAFFOLD DETOXICATION PROTOCOLS USING CELLTITER-GLO (PROMEGA, USA).

BOILING
UV LIGHT EXPOSURE
DIMETHYL SULFOXIDE SUBMERSION
VACUUM CHAMBER USE

PDGF-AB & IGF-I RELEASE

THE CONCENTRATION OF PDGF-AB AND IGF-I RELEASED AT CELL CULTURE MEDIUM BY IMBIBED SCAFFOLDS WERE MEASURED USING DUOSET ELISA (R&D SYSTEMS, USA) AT 5, 15 AND 30′ AND 1, 3, 6, 9 AND 24H.

PDGF-AB CONCENTRATION WAS ALSO ASSESSED AFTER ADDITION OF 50uM PAR1 TO PE.

RESULTS

ANOVA post-hoc Tukey.
Different letters indicate significant differences ($p<0.05$).

CELLULAR VIABILITY

Table 1. Non-post-treated 3D-printed discs.

	24 h (RLU)	48 h (RLU)	72 h (RLU)
Disc	41	121	54
Positive Control	2437	56000	1001
Negative Control	17	27	26

RLU: relative luminescence units.

Table 2. 3D-printed discs post-treated with different detoxication protocols.

	24 h (RLU)	48 h (RLU)	72 h (RLU)
Boiled	65.25 ± 2.5 A	51 ± 14.97 A	49.25 ± 9.29 A
DMSO	82.75 ± 7.04 A	51.25 ± 7.18 A	46 ± 14.74 A
O UV	63.5 ± 1.73 A	70 ± 7.96 A	88.5 ± 9.57 A
Positive Control	5925 ± 1779.04 B	10913 ± 4861.07 C	14681 ± 3699.65 D
Negative Control	38 ± 6.32 A	60.5 ± 8.54 A	92.5 ± 24.88 A

DMSO: dimethyl sulfoxide; O UV: overnight ultraviolet; RLU: relative luminescence units.

Table 3. 3D-printed scaffolds post-treated with different detoxication protocols.

	3 h (RLU)	24 h (RLU)
Dry VC	788.33 ± 11.01 A	6414 ± 124.13 B
VC+deionized water	809.5 ± 14.84 A	7477 ± 219.53 C
Positive Control	3111.66 ± 220.21 D	8035.16 ± 694.25 C
Negative Control	25.66 ± 2.88 A	47.66 ± 16.07 A

VC: vacuum chamber; RLU: relative luminescence units.

MIGRATION ASSAY

Table 4. Cell counting of 3D-printed scaffolds imbibed or not in PE-gel.

	24 h (#cells)	48 h (#cells)	72 h (#cells)
PE-gel+	49214.28 ± 909.13 A	38857.14 ± 2020.30 B	49214.28 ± 909.13 B
PE-gel -	100857.14 ± 1818.27 C	83000 ± 1010.153 D	100857.14 ± 1818.27 D

PDGF-AB AND IGF-I RELEASE AT CELL CULTURE MEDIUM BY PE-IMBIBED SCAFFOLD

Table 5. PDGF-AB concentration

	Scaffold (pg/mL)
5 min	213.31 ± 18.59 A
15 min	174.68 ± 16.90 A,B,D
30 min	160.18 ± 0.72 A,B,C,D
1 h	182.37 ± 29.21 A,B,D
3 h	133.33 ± 44.45 A
6 h	117.15 ± 19.91 B,C,D
9 h	87.45 ± 1.75 C,D
24 h	126.31 ± 0.78 D

Table 6. IGF-I concentration

	Scaffold (pg/mL)
5 min	0.161 ± 0.033
15 min	0.157 ± 0.038
30 min	0.105 ± 0.003
1 h	0.105 ± 0.054
3 h	0.140 ± 0.015
6 h	0.100 ± 0.024
9 h	0.073 ± 0.019
24 h	0.066 ± 0.048

Table 7. PDGF-AB concentration after different cold bath sonification intervals for PE-gel infusion.

	5 min (pg/mL)	10 min (pg/mL)
3 h	124.58 ± 2.7	115.52 ± 2.61
6 h	120.72 ± 0.33	101.33 ± 1.32
9 h	132.03 ± 7.48	121.32 ± 0.84
24 h	118.73 ± 2.52	125.04 ± 1.62

Table 8. PDGF-AB concentration using different designs.

	Design 1 (pg/mL)	Design 2 (pg/mL)
5 min	873.4811	650.1911
15 min	237.2763	273.7735
30 min	336.5915	174.2219
1 h	180.3332	30.97182
3 h	121.1824	0

Table 9. PDGF-AB concentration after the addition of PAR1.

	Scaffold (pg/mL)	Membrane (pg/mL)
5min	1.12 ± 2.24 A	235.37 ± 15.29 F
15min	9.61 ± 11.31 A	333.31 ± 27.07 B, G
30min	24.18 ± 26.18 A	284.97 ± 19.39 B, F
1h	38.30 ± 20.81 A	297.08 ± 17.50 B
3hrs	329.77 ± 43.96 B, G	415.48 ± 12.03 D, H
6hrs	486.08 ± 34.50 C, E	407.67 ± 13.44 D, H
9hrs	458.12 ± 37.15 C, D, E	386.46 ± 13.53 G, H
24hrs	515.48 ± 21.03 E	438.40 ± 12.57 C, H

CONCLUSIONS

- 3D printed polyurethane-based scaffolds are viable constructs for cell migration after a post-processing detoxification.
- The best protocol is the use of a vacuum chamber with the scaffold submerged at hot deionized water.
- The use of PE-gel seems to increase cell counting.
- The addition of PAR1 seems to be necessary to optimize platelet activation.

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