

Architecture-Promoted Biomechanical Performance-Tuning of Tissue-Engineered Constructs for Biological Intervertebral Disc Replacement

Gernot Lang ¹, Katja Obri ², Babak Saravi ^{1,3}, Aldo R. Boccaccini ², Anton Fröh ⁴, Michael Seidenstücker ⁴, Bodo Kurz ⁵, Hagen Schmal ¹ and Bernd Rolauffs ^{1,4,*}

¹ Department of Orthopedics and Trauma Surgery, Medical Center-Albert-Ludwigs-University of Freiburg, Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Hugstetterstrasse 55, 79106 Freiburg, Germany; gernot.michael.lang@uniklinik-freiburg.de (G.L.); babak.saravi@jupiter.uni-freiburg.de (B.S.); hagen.schmal@uniklinik-freiburg.de (H.S.)

² Institute of Biomaterials, Department of Material Science and Engineering, Friedrich-Alexander University of Erlangen-Nürnberg, Cauerstraße 6, 91058 Erlangen, Germany; katja.glier@web.de (K.O.); aldo.boccaccini@ww.uni-erlangen.de (A.R.B.)

³ AO Research Institute Davos, AO Foundation, Clavadelerstrasse 8, 7270 Davos, Switzerland

⁴ G.E.R.N. Research Center for Tissue Replacement, Regeneration & Neogenesis, Department of Orthopedics and Trauma Surgery, Medical Center-Albert-Ludwigs-University of Freiburg, Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Engesserstr 4, 79108 Freiburg im Breisgau, Germany; antonfrueh@aol.com (A.F.); michael.seidenstuecker@uniklinik-freiburg.de (M.S.)

⁵ Department of Anatomy, Christian-Albrechts-University, Otto-Hahn-Platz 8, 24118 Kiel, Germany; bkurz@anat.uni-kiel.de

* Correspondence: berndrolauffs@googlemail.com; Tel.: +49-761-270-26101

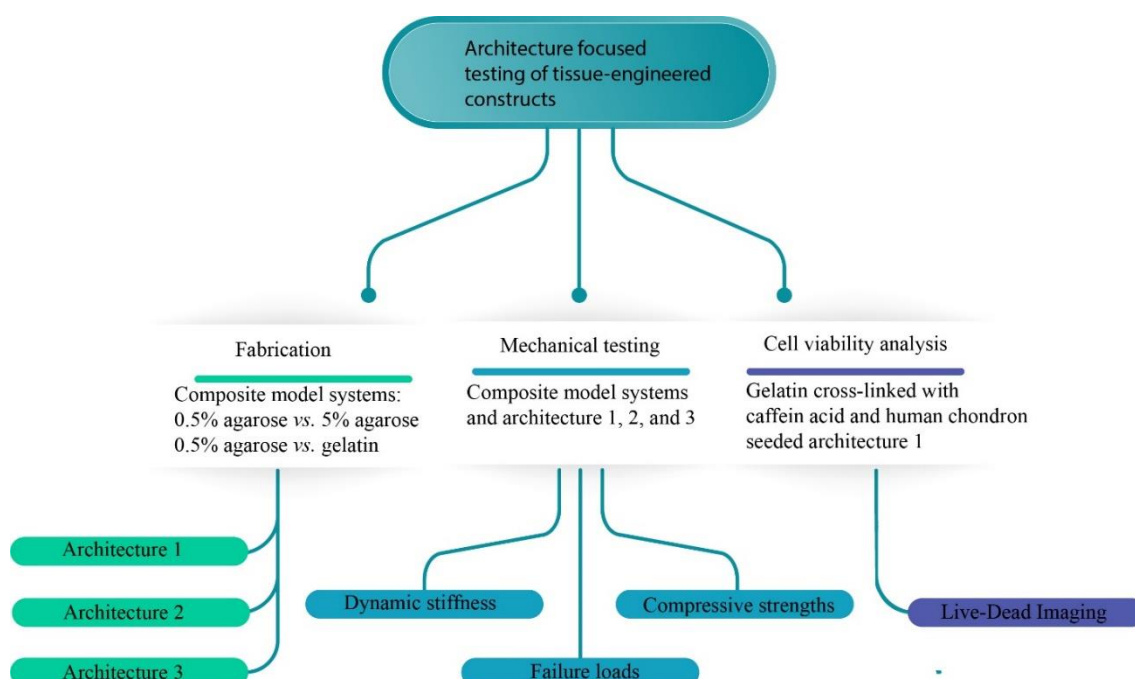


Figure S1. Experimental Setup.

Table S1. Composition of the 3D-life hydrogel.

Solution	Concentration	Volume [μ L]
H ₂ O		1.5
buffer solution (pH 5.5)		2.5
mal-Dextran		5
SH-peptide		10
cell suspension	16x10 ⁶ cells mL ⁻¹	5
PEG-Link		6

Table S2. Composition of the reagents for the 3D-life hydrogel.

Reagent	Composition
Mal-Dextran	30 mmol L ⁻¹ maleimide groups on dextran
	5 mmol L ⁻¹ phosphate buffer
PEG-link	20 mmol L ⁻¹ thiol groups on PEG
	10 g L ⁻¹ glucose
	0.5 mol L ⁻¹ 2-(N-morpholino)ethanesulfonic acid (MES)
	0.05 mol L ⁻¹ K Cl
	1.1 mol L ⁻¹ NaCl
buffer (pH = 5.5)	0.2 mol L ⁻¹ NaH ₂ PO ₄ 0.2 g L ⁻¹ phenol red pH-adjusted with HCl
cell suspension	chondrons in PBS (16 × 10 ⁶ cells mL ⁻¹)
water	H ₂ O (cell biology grade)

Table S3. Composition of the chondrocyte cultivation medium.

Solution	Supplier	Concentration [mg/mL]	Volume [mL]
Ham's F12 Nutrient Mix GlutaMax™	Life technologies		250
DMEM high glucose Gluta- Max™	Life technologies		250
Fetal Calf Serum (FCS)	BioChrom		50
Penicillin/Streptomycin	SigmaAldrich		10
Fungizone antimycotic	Life technologies		6
L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate	SigmaAldrich	25	0.5

Table S4. Composition of the digestion solution for chondron isolation.

Solution	Supplier	Concentration [u/mL]	Volume [mL]
Dispase 2	Roche	2.4	8
Collagenase P	Roche	1.5	2

Table S5. Composition of the digestion solution for chondrocyte isolation.

Solution	Supplier	Concentration [u/mL]	Volume [mL]
Dispase 2	Roche	2.4	8
Collagenase XI	SigmaAldrich	1500	2
Chondrocyte medium			8