

SUPPLEMENTARY MATERIAL

Title: The regenerative capacity of tissue-engineered amniotic membranes

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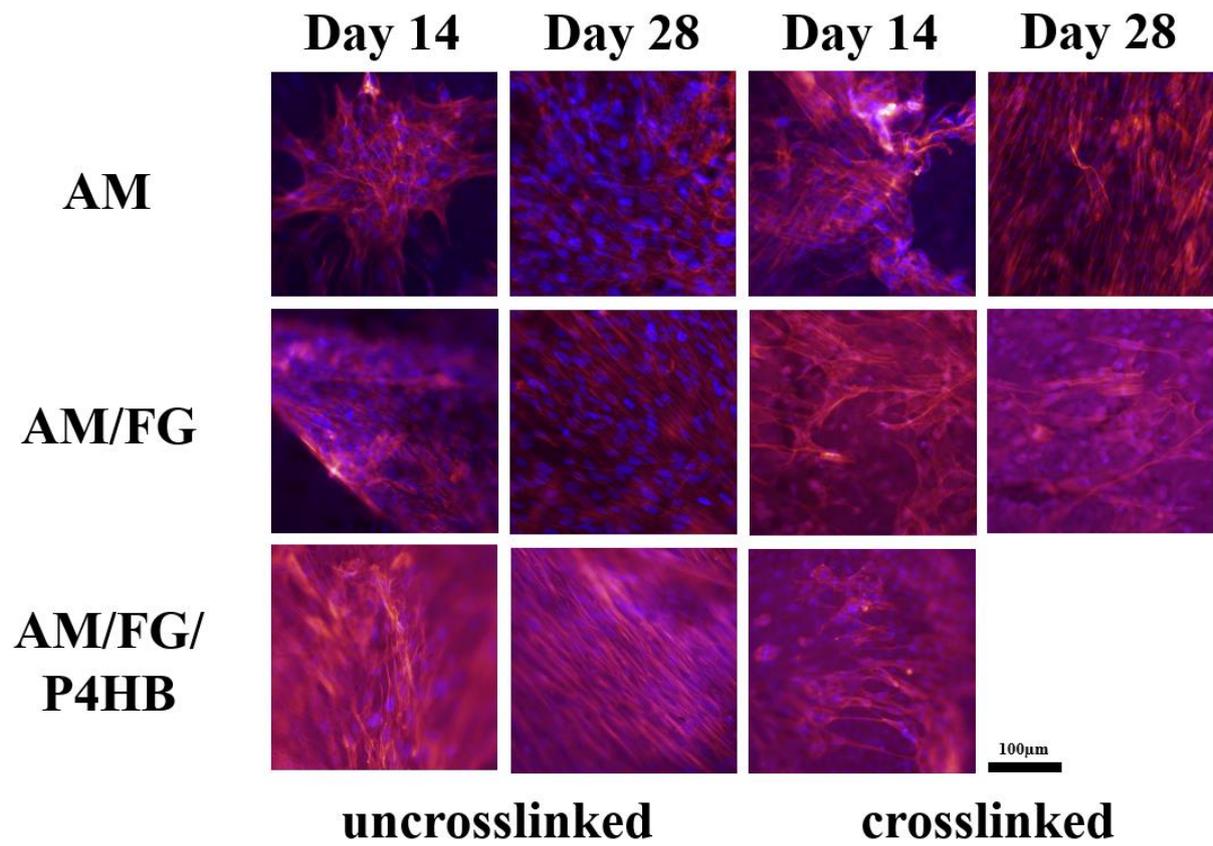
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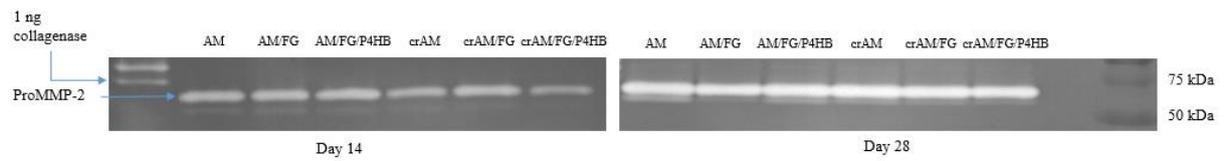
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Supplementary figure 1 – Fluorescent imaging.

Immunofluorescence of vaginal fibroblast at culture day 14 and 28. crAM/FG/P4HB at day 28 was not available.



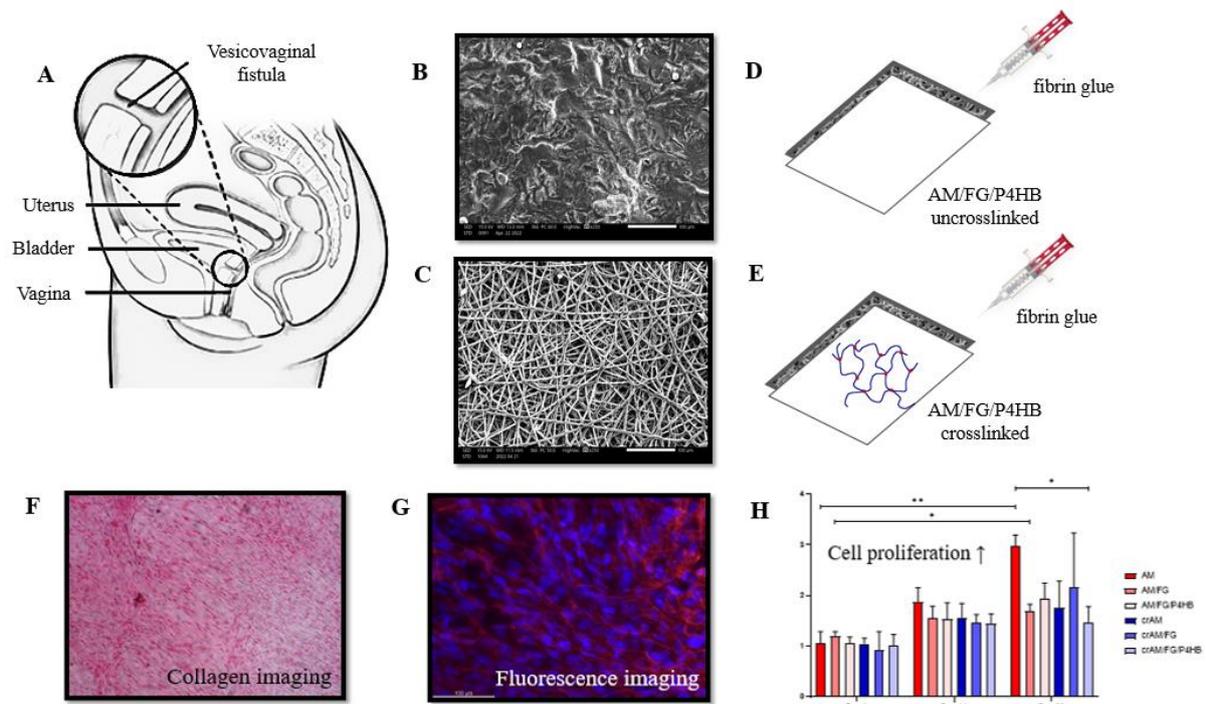
Supplementary figure 2: Zymography.

Example of zymography gel electrophoresis of proMMP-2 activity on day 14 and 28; on the left the reference categories with protein standard and 1ng collagenase. AM: uncrosslinked amniotic membranes; AM/FG: uncrosslinked amniotic membranes with fibrin glue; AM/FG/P4HB: uncrosslinked amniotic membranes with fibrin glue and electrospun poly-4-hydroxybutyrate (P4HB); crAM: crosslinked amniotic membranes; crAM/FG: crosslinked amniotic membranes with fibrin glue; crAM/FG/P4HB: crosslinked amniotic membranes with fibrin glue and electrospun poly-4-hydroxybutyrate (P4HB).

Supplementary table 1: Mechanical characteristics

Material		Tensile stress at break (MPa)	Maximum elongation (%)	Tangent modulus (MPa)	Maximum load (N)
Amniotic membranes	dry	10.5 (± 3.9)	2.5 (± 1.2)	5.63 (± 2.50)	1.1 (± 0.4)
	wet	3.8 [2.7-10.4]	12.5 (± 3.4)	0.81 (± 0.57)	0.4 [0.3-1.0]
P4HB (control)	P4-T0	4.5 (± 0.5)	112.3 (± 12.2)	0.31 (± 0.03)	19.1 (± 1.8)
	P4-T4	3.5 (± 0.8)	74.7 (± 26.1)	0.48 (± 0.05)	14.7 (± 3.5)
	P4-T8	3.6 (± 0.4)	64.0 (± 15.6)	0.62 (± 0.11)	16.5 (± 2.5)
P4HB + fibrin glue	P4FT0	2.7 (± 0.3)	66.6 (± 7.2)	0.23 (± 0.02)	13.7 (± 1.4)
	P4FT4	1.6 (± 0.5)	24.1 (± 12.8)	0.37 [0.36-0.45]	6.6 (± 1.6)
	P4FT8	0.8 (± 0.3)	9.4 (± 2.6)	0.29 (± 0.06)	4.0 (± 1.6)
P4HB + fibrin glue + AM	P4AT0	2.8 (± 0.2)	70.1 (± 5.6)	0.21 (± 0.02)	14.6 (± 1.2)
	P4AT4	2.6 (± 0.2)	59.4 (± 2.0)	0.37 (± 0.05)	12.2 (± 0.7)
	P4AT8	1.0 (± 0.2)	12.5 (± 2.4)	0.30 (± 0.08)	5.0 (± 0.8)

Data is reported as mean (\pm standard deviation) or median [25th – 75th percentile]; All samples had width of 10mm;
AM: amniotic membrane; *P4HB*: poly-4-hydroxybutyrate; *cxl*: crosslinking experiment; P4-: P4HB control sample; P4F: P4HB with fibrin glue;
P4A: P4HB with AM glued together with fibrin glue; T0: baseline measurement; T4: degradation 4 weeks; T8: degradation 8 weeks;



A: Schematic representation of a vesicovaginal fistula; B: Scanning electron microscopy (SEM) image of amniotic membranes at $\times 250$ -magnification; C: SEM image of poly-4-hydroxybutyrate (P4HB) at $\times 250$ -magnification; D: Preparation of P4HB samples: P4HB scaffold and uncrosslinked AM (white sheet) glued together with fibrin glue; E: Preparation of P4HB samples: P4HB scaffold and crosslinked AM (white sheet with crosslinking network) glued together with fibrin glue; F: Example of collagen imaging; G: Example of fluorescence imaging; H: Graph with cell proliferation data.