Engineering Human Dermal Fibroblasts with Impaired Migration for In Vitro Models of Aged Wound Healing: Testing of New Biomaterial-Based Therapies for Chronic Wounds

Elena García-Gareta[1], Alexandra Levin[1], Lilian Hook[2]

[1]Regenerative Biomaterials Group, RAFT Institute, Mount Vernon Hospital, Northwood, UK

[2]Smart Matrix Ltd, Leopold Muller Building, Mount Vernon Hospital, Northwood, UK

Background: The geriatric population is prone to chronic wounds, which cost the UK National Health System £4 billion/year. Current treatments are inefficient. Aged dermal fibroblasts, usually found in chronic wounds, attach normally but show deficient migration due to a significant reduction in α2β1 integrin function, although α2 integrin expression is normal [1].

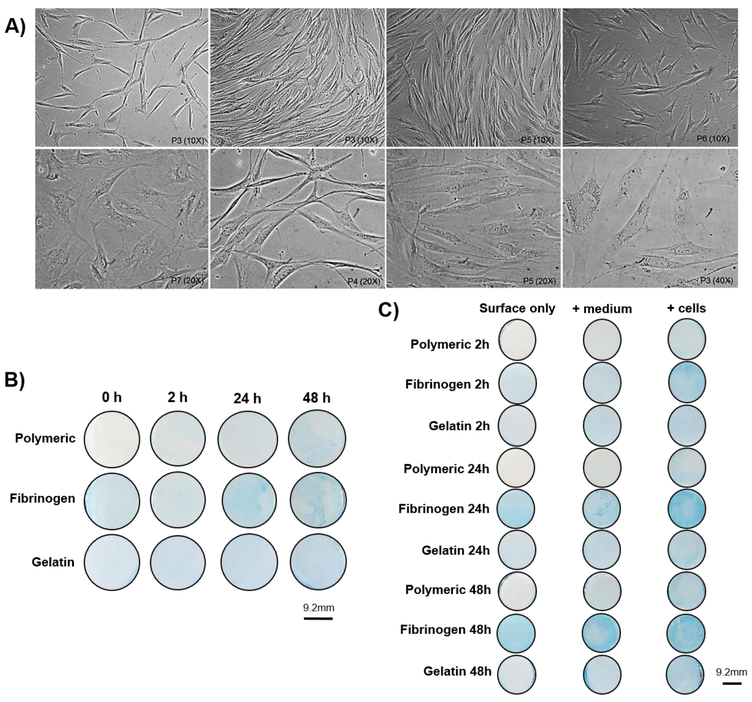
We report the engineering of a population of cells that mimicked the behaviour of aged dermal fibroblasts, that could be used in in vitro models of aged wound healing to test new biomaterial-based therapies. We used synthetic RGD peptides.

Aim: to engineer a population of cells that mimic the behaviour of aged dermal fibroblasts found in chronic wounds

Method & Results

Cells: primary normal human dermal fibroblasts (pnHDFs) from a single donor (A).

2D Surfaces: The first part of our study was carried out on 3 different 2D surfaces representative of the materials used to develop dermal scaffolds: 1) hydrophilic synthetic polymer, 2) fibrinogen and 3) gelatin. All surfaces contained RGD binding sites from serum in medium and cellular ECM deposition (B, C).



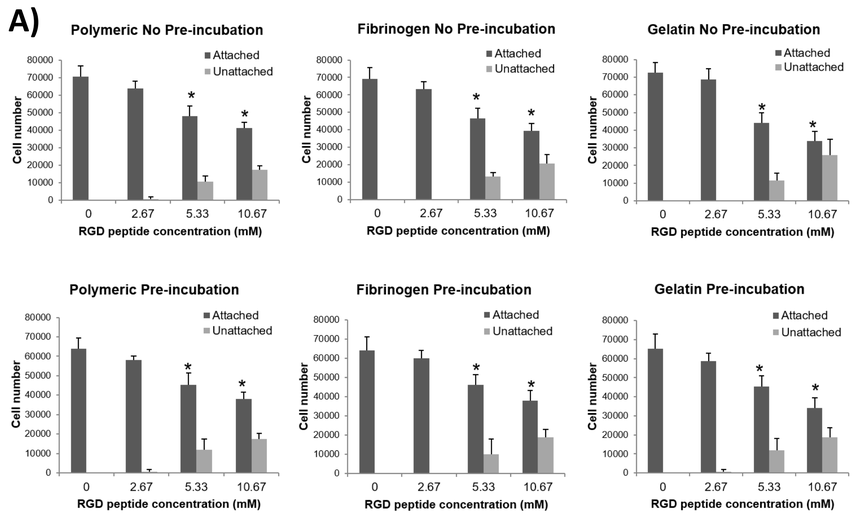
3D dermal scaffolds: the commercially available Integra® and Matriderm® were chosen as they are made of dermal ECM components (mostly collagen). The mechanism of action of both scaffolds is through integrins' ligands.

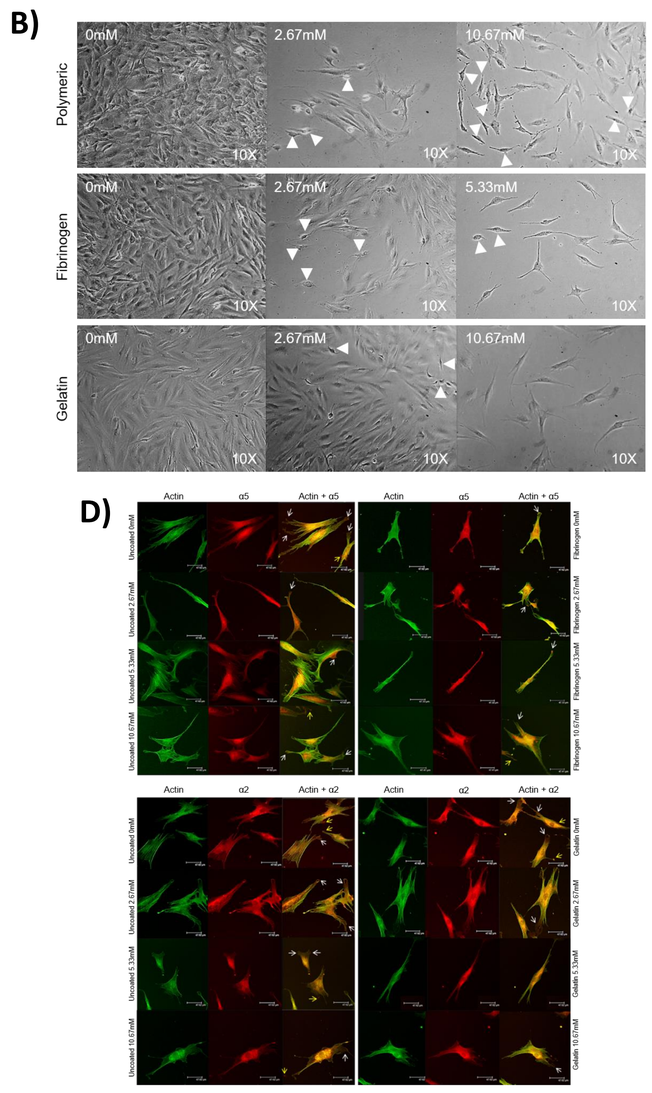
Results in 3D scaffolds showed that the concentration of synthetic RGD peptides necessary to impair migration of dermal fibroblasts should be tailored to the number of RGD sites present in the 3D matrix.

Cell attachment on 2D surfaces was reduced in a concentration dependent manner (A, *p<0.05). Viability was ≥ 96.25%. In the absence of RGD peptides cells formed a confluent monolayer, but as peptides were added the monolayer was disrupted and cells had a more rounded morphology (B, white arrows).

Cell migration: cells cultured on gelatin surfaces looked the most spread and obtained overall the least reduction in cell migration (C), suggesting that the number of RGD sites of the surface may influence the effect of the peptides.

Expression of integrins was not affected and the components of the migration pathway were not altered by the peptides (D).

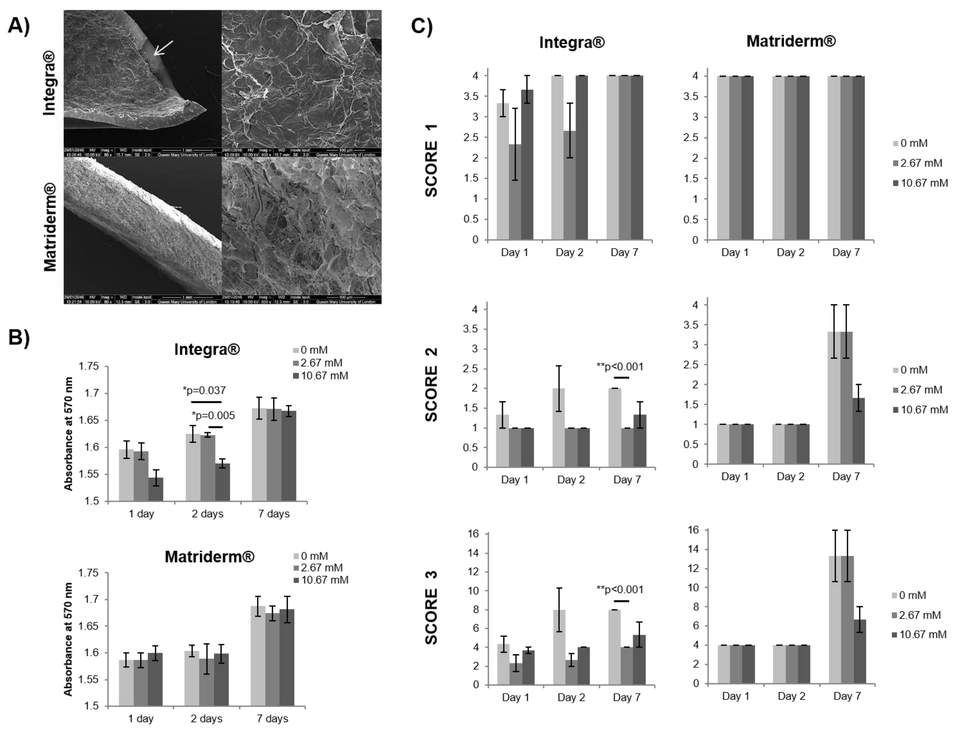




C)

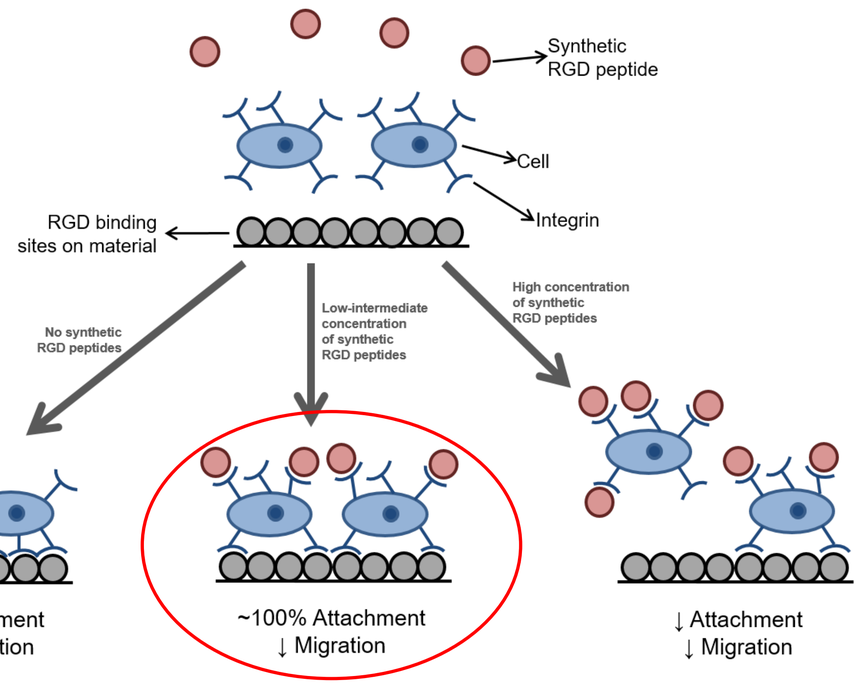
Conditions of cell migration experiment	RGD concentration (mM)	Polymeric	Fibrinogen	Gelatin
- Cells not pre-incubated with RGD peptides before seeding. - Addition of RGD peptides after creating the scratch.	0			
	2.67			
	5.33			
	10.67			
- Cells pre-incubated with RGD peptides before seeding. - No addition of RGD peptides after creating the scratch.	0			
	2.67			
	5.33			
	10.67			
- Cells pre-incubated with RGD peptides before seeding. - Addition of RGD peptides after creating the scratch.	0			
	2.67			
	5.33			
	10.67			

Average Score	6 – 5	4.99 - 4	3.99 - 3	2.99 - 2
Colour Key				



Summary:

- Low-intermediate concentrations of RGD peptides do not block a significant percentage of the integrins and therefore, cells are able to attach but their migration is reduced.
- Low to intermediate concentrations, which will depend on the number of RGD sites on the surface or 3D matrix, should be used to mimic the behaviour of aged dermal fibroblasts.



Conclusion:

- Using synthetic RGD peptides, we engineered a cellular population that mimics the behaviour of aged dermal fibroblasts, usually found in the clinically challenging chronic wounds.
  - This technology could be translated to other cell types including established cell lines, thus eliminating the need for primary tissue harvest.

References & Acknowledgements:

[1] M.J. Reed et al. Mech Ageing Dev 122(11), 1203, 2001.

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