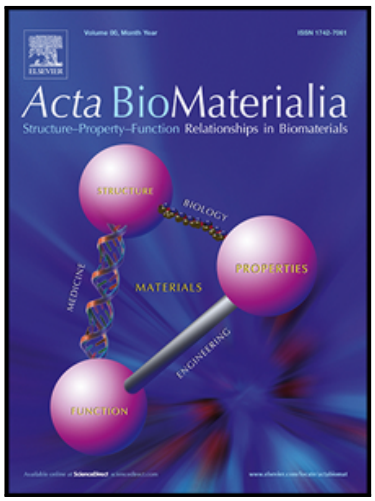

Host macrophage response to injectable hydrogels derived from ECM and α-helical peptides

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Host macrophage response to injectable hydrogels derived ŦN2Y 9/ a l·yŘ h-helical peptides

Authors

Nazia Mehrban[1*], Catalina Pineda Molina[2, 7], Lina M. Quijano[2, 7], James Bowen[3], Scott A. Johnson[2, 7], Joseph Bartolacci[2, 8], Jordan T. Chang[2], David A. Scott[4], Derek N. Woolfson[4, 5, 6], Martin A. Birchall[1], Stephen F. Badylak[2, 7, 8]

[1]McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3110, USA, Permanent Address: UCL Ear Institute, University College London, 332 Grays Inn Rd, London, WC1X 8EE, UK, [2]McGowan Institute for Regenerative Medicine, University of Pittsburgh, 450 Technology Drive, Suite 300, Pittsburgh, PA 15219-3110, USA, [3]School of Engineering & Innovation, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK, [4]School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK, [5]School of Biochemistry, University of Bristol, University Walk, Bristol, BS8 1TD, UK, [6]Bristol BioDesign Institute, University of Bristol, 24 Tyndall Avenue, Bristol, BS8 1TQ, UK, [7]Department of Surgery, School of Medicine, University of Pittsburgh, University of Pittsburgh Medical Center Presbyterian Hospital, 200 Lothrop Street, Pittsburgh, PA 15213, USA, [8]Department of Bioengineering, University of Pittsburgh, 3700 O'Hara Street, Pittsburgh, PA, 15261, USA

∗Corresponding author. N. Mehrban (e-mail: n.mehrban@ucl.ac.uk); Permanent Address: UCL Ear Institute, University College London, 332 Grays Inn Rd, London, WC1X 8EE, UK; +44 (0) 203 456 7870.

Abstract

Tissue engineering materials play a key role in how closely the complex architectural and functional characteristics of native healthy tissue can be replicated. Traditional natural and synthetic materials are superseded by bespoke materials that cross the boundary between these two categories. Here we present hydrogels that are derived from decellularised extracellular matrix and those that are synthesised from *de novo* h-helical peptides. We assess *in vitro* activation of murine macrophages to our hydrogels and whether these gels induce an M1-like or M2-like phenotype. This was followed by the *in vivo* immune macrophage response to hydrogels injected into rat partial-thickness abdominal wall defects. Over 28 days we observe an increase in mononuclear cell infiltration at the hydrogel-tissue interface without promoting a foreign body reaction and see no evidence of hydrogel encapsulation or formation of multinucleate giant cells. We also note an upregulation of myogenic differentiation markers and the expression of anti-inflammatory markers Arginase1, IL-10, and CD206, indicating pro-remodelling for all injected hydrogels. Furthermore, all hydrogels promote an anti-inflammatory environment after an initial spike in the pro-inflammatory phenotype. No difference between the injected site and the healthy tissue is seen after 28 days, indicating full integration. These materials offer great potential for future applications in regenerative medicine and towards unmet clinical needs.

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Materials play a key role in how closely the complex architectural and functional characteristics of native healthy tissue can be replicated in tissue engineering. Here we present injectable hydrogels derived from decellularised extracellular matrix and *de novo* ŘŜăƛƎyŜŘ h-helical peptides.

Over 28 days in rat abdominal wall we observe an increase in mononuclear cell infiltration at the hydrogel-tissue interface with no foreign body reaction, no evidence of hydrogel encapsulation and no multinucleate giant cells. Our data indicate pro-remodelling and the promotion of an anti-inflammatory environment for all injected hydrogels with evidence of full integration with healthy tissue after 28 days. These unique materials offer great potential for future applications in regenerative medicine and towards designing materials for unmet clinical needs.

Keywords: hydrogels; biomaterials; peptide; ECM; macrophage

1. Introduction

The repair or replacement of damaged tissue using tissue engineering strategies is strongly influenced by the material selected to mimic the architecture and functional characteristics of native healthy tissue. Published sources of bespoke materials are either biologically derived or synthetic.[1, 2] *In vitro* response to these biomaterials are usually assessed by their ability to promote a specific cell responses. However, it is the host immune response to implanted biomaterials that is the critical, if not defining, determinant of clinical success or failure.[3, 4] A desirable host response relies on cell infiltration and material integration/remodelling in support of an optimal functional outcome. Prolonged inflammation typically results in the formation of granulation tissue or fibrous capsules,[5] seroma, scars and encapsulation.[6] Such an effect results in the isolation of the implanted material from the surrounding healthy tissue and prevents the formation of new functional tissue. Macrophages represent a major cellular component of the innate immune response to biomaterials. These cells show diverse plasticity in their functions ranging from pro-inflammatory to anti-inflammatory and reparative phenotypes.[7, 8]

Contrary to accepted 25-year dogma, macrophages, among other cell types such as muscle-specific regulatory T cells and satellite cells,[9] are essential for normal tissue development.[10-13] Macrophages are necessary for successful tissue and organ regeneration in regenerative species such as the axolotl,[14, 15] and have the ability to affect stem cell/ progenitor cell differentiation,[16] and proliferation óλΦŜΦΣ Ι·ℕŜ yƲű yŜŐŜăăΙ·ℕλfê άŜyŘ ăűΙ·ΞŜε ŐŜffăúΦ[17] Given this relatively recent understanding of macrophage biology and their role in critical life processes, the signalling molecules, physical factors, and environmental factors that influence macrophage phenotype are of great interest to the biomaterials community.

Naturally occurring biomaterials composed of extracellular matrix (ECM), such as decellularised tissues[18-20], have been shown to contain a variety of potent signalling molecules that are released or exposed during degradation of the matrix. These include cryptic peptides,[14] cytokines and chemokines,[20, 21] and, most recently, embedded matrix bound nanovesicles (MBV).[22] In addition to the chemical cues, these materials provide physical signals such as material stiffness, pore size, and load transfer,[20] which have all been shown to influence macrophage phenotype.[23] Several tissue types have been decellularised and used either in their original form, as sheets[24], or as injectable hydrogels.[20] The tissues used include dermis,[25, 26] small intestinal submucosa (SIS),[27-29] urinary bladder matrix (UBM),[30, 31] liver,[32] tendons,[33] and whole limbs[34]. The use of decellularised ECM in clinical applications is commonplace with several FDA-approved ECM products currently available on the ΥΙ·ℕ]Ŝű λyŐfdzŘλyΞ !ff25ŜℕΥ†Σ bŜ2C2ℕΥ𝜅Σ DℕΙ·ŦűWΙ·Ŏ]Ŝű†Σ {úℕΙ·úűλŐŜ𝜅Σ aŜă2 .λ2Matrix®, SynerGraft® Oasis® and Surgisis®.

There have been many attempts to mimic properties of the native ECM within synthetic biomaterials.[35-37] It is possible to study individual ECM proteins and to design peptides that are

capable of hydrogel configurations.[38-42] Some aspects of the complexity of naturally occurring ECM can be incorporated through the addition of cell-guiding chemical moieties.[43-45] The use of synthetic peptides to accomplish these ends is not new, and there are several reports of natural proteins that are used to create hydrogels suitable for clinical applications.[46-48] The benefits of previously described hydrogelating self-assembling fibre (hSAF) systems include their stable ^{h}-helical structure, modifiable chemistry and ability to form at low concentrations compared to other peptide systems.[42, 43, 49] Briefly, the hSAF system is formed from two complementary *de novo* designed ^{h}-helical peptides. The two peptide sequences form sticky-ended dimers that assemble end-to-end to form fibres. At mM concentrations, the mixed hSAF peptide fibres form self-supporting hydrogels. Combined with added chemical functionality, such as the addition of adhesive ligands, these gels offer at least partial control over cell adhesion, migration and differentiation[43], all characteristics of a material with high clinical translational potential.

While there are clear compositional differences between SIS, UBM and hSAFs, they all form hydrogels with networks through which cells can migrate and form 3-dimensional constructs. Herein, we investigate the macrophage response to injectable UBM, SIS, bovine collagen (type I; FibriCol) and hSAF hydrogels in a partial thickness abdominal wall defect model, in Sprague Dawley rats over 28 days, using the native tissue for comparison. Macrophage interaction with these materials will provide a pre-clinical indicator of the likely cell response *in vivo* and offer insights into strategies for promoting cell infiltration and promoting functional restoration of damaged tissue.

2. Materials and Methods

2.1 Material Preparation

2.1.1 Small Intestinal Submucosa

Porcine small intestinal submucosa (SIS) was prepared using previously described methods [50]. Briefly, the intestine was flushed with double distilled water, opened horizontally and most of the tunica mucosa, and entirety of tunica serosa and tunica muscularis externa were removed using a scraper. The remaining tunica submucosa and basilar layers of the tunica mucosa were cut to 80 g particles and shaken at 300 rpm for 2 hrs at room temperature in 0.1% peracetic acid (v/v) (Rochester Midland, USA) with 4% ethanol (v/v) (Decon Laboratories, USA) in Type I H_2O. Tissue was washed thrice in Phosphate Buffered Saline (PBS; Fisher Scientific, USA) and sterile water and shaken at 300 rpm for 15 mins each before being frozen overnight at -20°C, cut into 1 cm x 1 cm sections, lyophilised and milled using a 60 µm mesh on a Wiley Mill (GE Motors & Industrial Systems, USA). Milled small intestinal submucosa (SIS) was stored at room temperature in the dark until required.

2.1.2 Urinary Bladder Matrix

Urinary bladders were harvested from market-weight (N240 lbs.) pigs (Animal Biotech Industries, USA). The bladders were decellularised as previously described [51]. Briefly, they were rinsed using double distilled water and the urethra and ureter removed. The bladders were opened along their length and a bevelled scraper was used to stretch the muscle across the abluminal surface of the tissue. The tunica serosa, tunica muscularis externa, tunica submucosa and muscularis mucosa were gently removed by making a shallow incision along the length of the bladder (from the apex to the neck). The remaining tunica propria and basement membrane were submerged in double distilled water to reveal any remnant muscle fibres. These remaining fibres were removed and urinary bladder matrix (UBM) was cut to 80 g particles and shaken at 300 rpm for 2 hrs at room temperature in 0.1% peracetic acid (v/v) with 4% ethanol (v/v) in Type I H_2O. The UBM was then washed thrice in PBS and sterile water on an orbital shaker at 300 rpm for 15 mins each before being frozen overnight at -20°C, cut into 1 cm x 1 cm

sections, lyophilised and milled using a 60 µm mesh on a Wiley Mill. Milled UBM was stored at room temperature in the dark until required.

2.1.3 hSAF Peptides

Two complementary peptides (hSAF-p1 and hSAF-p2) were synthesised as previously described.[49] Briefly, the peptides were synthesised on a 0.1 mmol scale by solid phase peptide synthesis on a CEM á[λõŜℕűề .ŧdzŜε YʹλŎℕ2ø⊦ØŜ-assisted peptide synthesiser (CEM, USA) using fluorenylmethyloxycarbonyl (Fmoc) chemistry. After cleavage from the resin, the peptides were purified by reverse-phase HPLC (Jasco, UK) on a Luna C18 column (5 µm, 100 Å, 4.6 mm x 150 mm ID), and their masses confirmed by MALDI-TOF mass spectrometry (Applied Biosystems 4700 Proteomics MALDI-TOF Analyzer). Peptides were then lyophilised and stored at 4°C until required.

2.2 Hydrogel Preparation

For SIS and UBM the lyophilised powders were first digested using 1 mg/mL pepsin (Sigma, USA) in 0.01 N HCl for 48 hrs at room temperature whilst continuously stirring. Digested SIS and UBM were neutralised using 0.1 N NaOH and PBS to concentrations of 8 mg/mL (SIS) and 15 mg/mL (UBM). FibriCol hydrogels (Advanced Biomatrix, USA) were formed according to mayðzŦ⊦ŐűdzℕŜℕΩă ʎyãűℕdzŎűʎ2yã ű2 ⊦ concentration of 8 mg/mL. 1 mM hSAF hydrogels were produced using previously described methods.[43]

2.3 Bone Marrow-derived Macrophage Isolation and Culture

Bone marrow-derived mononuclear cells were obtained from female C57bl/6 mice (Jackson Laboratories, USA) and differentiated into macrophages using a previously described technique [29]. In õℕλŜŦΣ űKŜ ŦŜYʹdzℕ ⊦yŘ űʎõʎ⊦ øŜℕŜ ʎă2f⊦űŜŘ ⊦yŘ ø⊦ăKŜŘ ʎyʹ ăʹdzLJLJfŜYʹŜyűŜŘ 5ʹdzfõŜŎŎ2Ωă a2ŘʎŦλŜŘ 9⊦ƎfŜΩă

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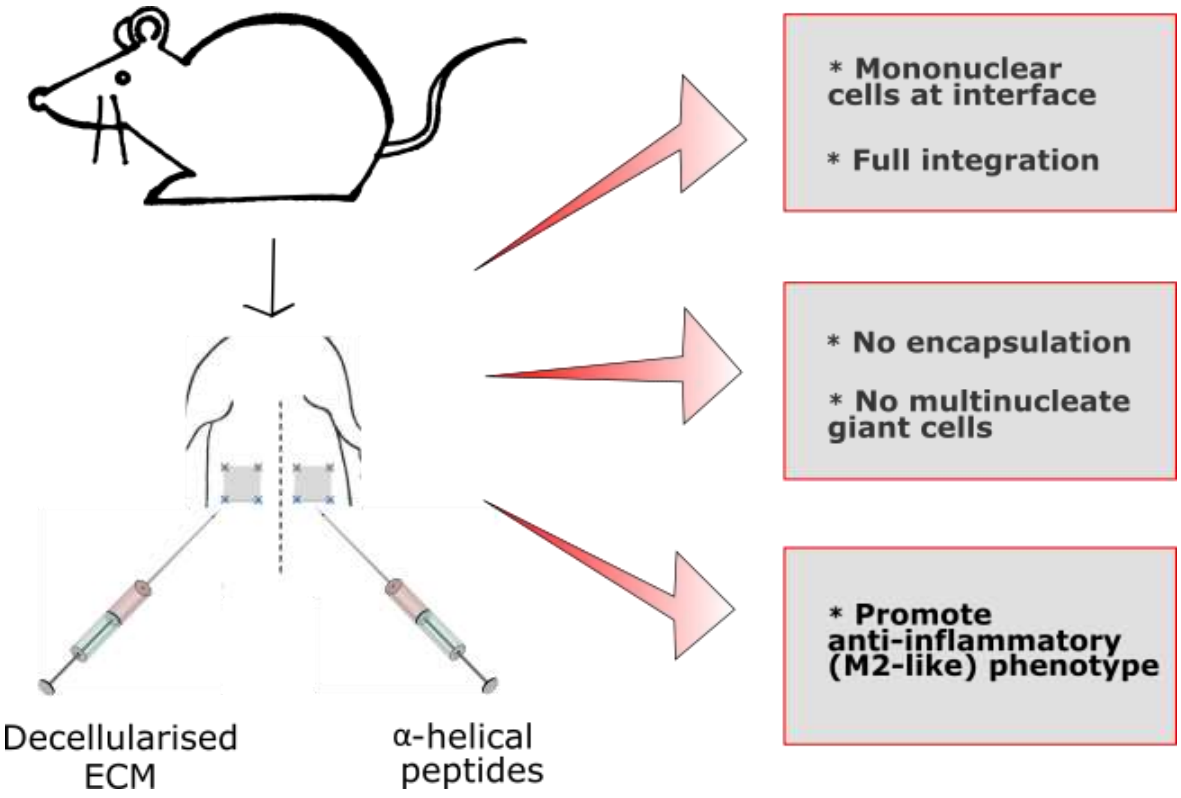
Graphical Abstract



Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: