

COMPARATIVE INVESTIGATION OF SEVEN CANDIDATE SCAFFOLDS FOR THE PRODUCTION OF AN AUTOLOGOUS TISSUE ENGINEERED CONNECTIVE TISSUE FOR USE IN STRESS URINARY INCONTINENCE AND PELVIC ORGAN PROLAPSE

Hypothesis / aims of study

Our aim was to assess seven different scaffolds; Alloderm (AL), cadaveric dermis (CD), polypropylene (PPL), sheep forestomach (SF), porcine dermis (PD), porcine small intestinal submucosa (SIS) and thermoannealed poly(L)-lactic acid (Th PLA) for potential use as a scaffold for the attachment of fibroblasts to create a tissue engineered connective tissue suitable for use in SUI and POP.

Study design, materials and methods

We expanded oral mucosal fibroblasts obtained with full patient consent and ethical approval. Fibroblasts (800 000) were seeded onto 4cm² pieces of the seven different matrix materials and were cultured for a period of two weeks in 10% DMEM medium. The tissue engineered matrices were assessed for:

- 1) Cell attachment using AlamarBlue (a vital stain) and DAPI (a nuclear stain)
- 2) Total collagen production as assessed by Sirius red staining
- 3) Collagen types I,III, IV and elastin as assessed by immunostaining
- 4) Extracellular matrix production as assessed by scanning electron microscopy (SEM)

Results

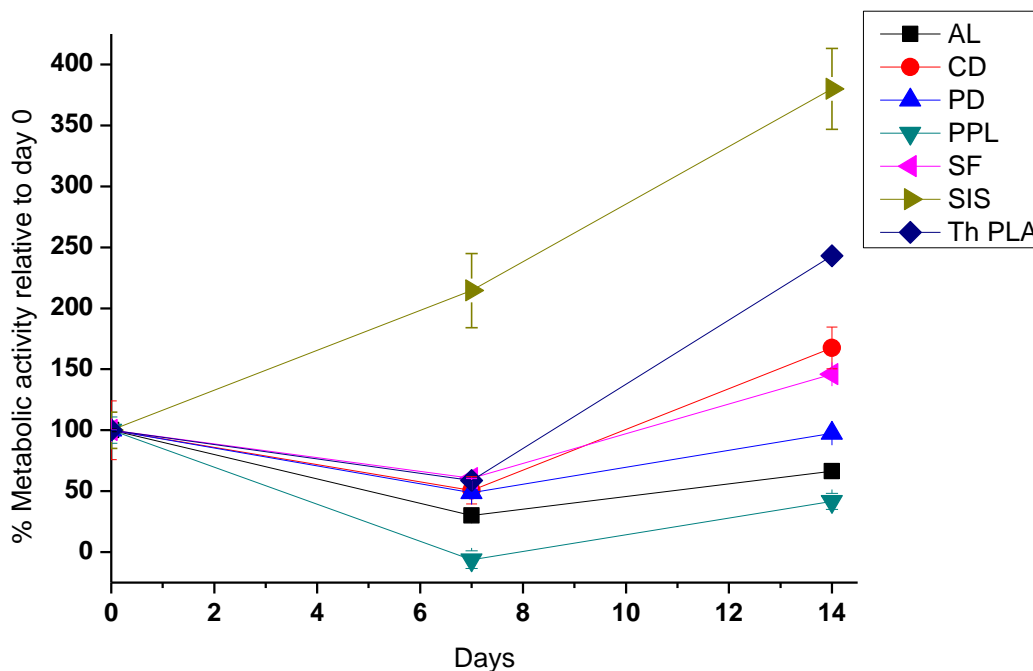


Fig 1. Metabolic activity of cells on the different scaffolds over 14 days relative to day 0, n=9±SEM

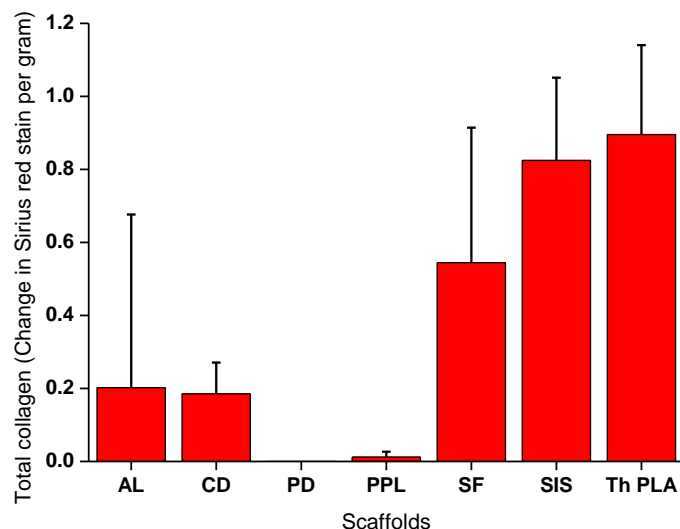


Fig 2. Production of collagen by cells on scaffolds over 14 days culture, n=9±SEM

Material	Collagen I	Collagen III	Collagen IV	Elastin	Extracellular matrix
AL	+	+	-	+	+
CD	+	-	-	-	+
PD	-	-	-	-	+/-
PPL	-	-	-	-	-
SF	+	-	-	+	+/-
SIS	++	+	-	++	+
Th PLA	++	+	-	+++	+++

Table 1. Production of collagens I, III, IV and elastin assessed by immunofluorescence and total matrix production by SEM. (A qualitative scale of: - absent, +/- equivocal, + some, ++ good, +++ abundant)

Interpretation of results

Fig 1. Shows that the metabolic activity of cells on the scaffolds was greatest on SIS followed by Th PLA. This increased relative to day 0. DAPI (images not shown) and Sirius red staining (Fig 2) revealed this was due to cell proliferation and collagen/ elastin production. Fibroblasts produced collagen I, III and elastin (Table 1). SEM revealed abundant new extracellular matrix on Th PLA and a good matrix on SIS.

Concluding message

These results show that both Th PLA and SIS are good scaffolds which support fibroblasts and allow the production of a new connective tissue matrix containing collagen I, III and elastin *in vitro*. We conclude that both should now be assessed in animal studies to assess tissue integration and vascularisation.

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Is this a clinical trial?	No
What were the subjects in the study?	NONE