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Mangera A[1], Bullock A J[2], MacNeil S[2], Chapple C[1]
1. Sheffield teaching hospitals NHS trust, 2. University of Sheffield

DEVELOPING AN AUTOLOGOUS TISSUE ENGINEERED PROSTHESIS FOR USE IN STRESS URINARY INCONTINENCE AND PELVIC ORGAN PROLAPSE.

Hypothesis / aims of study
Our aim is to develop a tissue engineered mesh suitable for use in SUI and POP using a scaffold support and patients' buccal fibroblasts. In this study we compared four potential scaffolds for their ability to support cell attachment and we examined their mechanical properties both with and without cells and the ability of cells to remodel these scaffolds by producing collagen. The scaffolds were cadaveric dermis (CD), polypropylene (PPL), porcine small intestinal submucosa (SIS) and thermoannealed poly(L)-lactic acid (Th PLA).

Study design, materials and methods
We obtained and expanded fibroblasts from oral mucosal biopsies. 800 000 fibroblasts were attached to each $2cm^2$ of matrix. These were cultured for a period of two weeks in 10% DMEM medium. The tissue engineered prostheses were assessed for:
1) Cell attachment using AlamarBlue (a vital stain) and DAPI (a nuclear stain)
2) Cell mediated contraction as assessed by serial photographs
3) Mechanical properties; including ultimate tensile stress and Young's modulus of elasticity using a Bose electroforce instrument
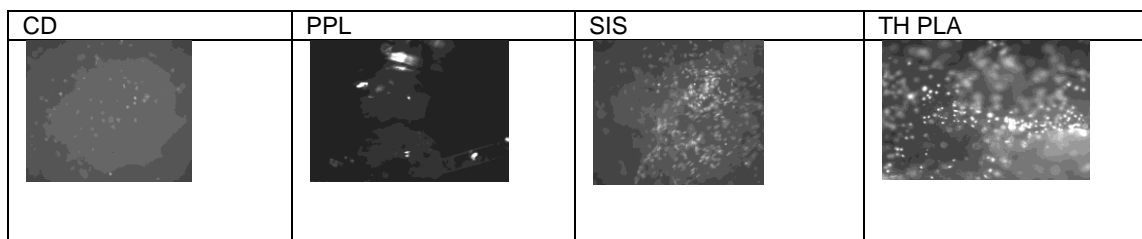4) Collagen production as assessed by Sirius red staining

Results

Fig 1. DAPI Images of cells cultured within the different scaffolds using Fluorescence microscopy (X100)

Scaffold	Relative cellular activity (AlamarBlue) at day 14 compared to day 0 (Seeded with 800 000 cells = 100%) (n=6+SEM)	Cell mediated contraction after 14 days culture (n=6+SEM)
CD	197%	18.17% (+3.5)
PPL	38%	1.34% (+0.35)
SIS	453%	14.2% (+1.64)
Th PLA	274%	17.8% (+5.79)

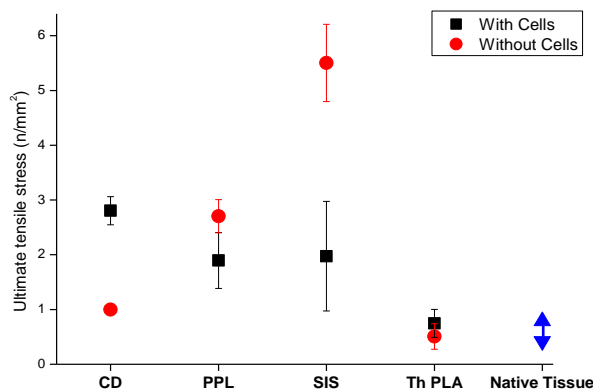Table 1. Relative cellular activity and cell mediated contraction of scaffolds at 14 days

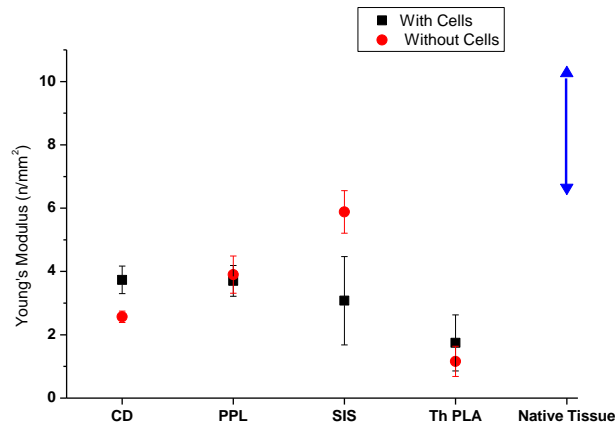Fig 2.Ultimate tensile strength of scaffolds with and without cells compared to native tissue (1), (n=3 +SEM)

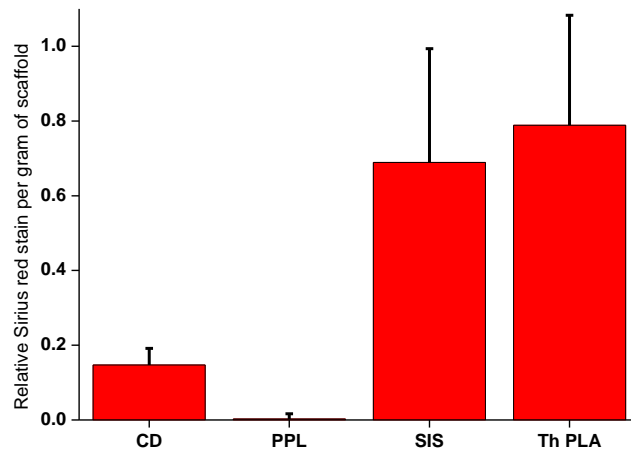Fig 3. Young's Modulus of elasticity of scaffolds with and without cells compared to native tissue (1) (n=3 $\pm$SEM)

Fig 4. Collagen Production of cells in scaffolds as assessed by Sirius red staining (n=6$\pm$SEM)

Interpretation of results
We found poor cellular activity and attachment (Fig 1) of fibroblasts to macroporous polypropylene resulting in poor contraction and collagen production. CD, SIS and Th PLA showed good cellular activity and cell mediated contraction. Compared to the mechanical properties of the native tissue (see Fig 2 & 3) which represent the ideal mechanical values for paravaginal tissue, many of the scaffolds were stronger than these tissues but with a lower modulus of elasticity. Further work therefore needs to be concentrated on achieving this. However, encouragingly the ability of cells to produce collagen was very good for cells cultured on SIS and Th PLA.

Concluding message
Of the three materials, PPL is very poor as a candidate scaffold material. The other three have potential, of which SIS and TH PLA were the best. These latter scaffolds support cell attachment and collagen synthesis and with further work offer the potential to be developed as an autologous tissue engineered scaffold for clinical use.

References
1. (1) Lei L, Song Y, Chen R. Biomechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18:603-607.

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