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DEVELOPING AN AUTOLOGOUS ENGINEERED CONNECTIVE TISSUE USING A BIODEGRADABLE SCAFFOLD FOR THE TREATMENT OF STRESS URINARY INCONTINENCE AND PELVIC ORGAN PROLAPSE

Hypothesis / aims of study

Stress urinary incontinence (SUI) and pelvic organ prolapse (POP) are very bothersome diseases with a huge and rising prevalence in women from all ages (1). Currently there is no recommended material for use in the surgical management of these disorders. Synthetic and biological materials are all used as cell free prosthesis and are often degraded and their biomechanical properties deteriorate with time producing worrying failure and erosion rates (2).

Our aim is to develop a cell impregnated scaffold to achieve a repair material to treat SUI and POP, which will achieve long lasting repair by good integration into the native tissues maintaining the biomechanical properties with time by renewal of extracellular matrix (ECM) components.

Study design, materials and methods

Tissue engineered prosthesis were developed from comparing oral mucosal fibroblasts (OFs) and adipose-derived stem cells (ADSCs) for cell attachment and proliferation, ECM production and biomechanical properties under free, static and dynamic culture conditions.

Isolation and culture of human OFs were obtained from oral mucosal biopsies and human ADSCs from subcutaneous fat, using mechanical and enzymatic procedures.

Th PLA (Thermoannealed poly-(L)-lactic acid) was chosen as our candidate scaffold, being a biodegradable material which was made via electrospinning technique in a clean room.

OFs, ADSCs, and a mixture of both cells were seeded at a density of one million cells per scaffold (2-3cm^2) and cultured in DMEM media supplemented with 10% FCS on Th PLA for two weeks. Each scaffold was either kept in unrestrained (free condition), restrained using plastic rings (static condition), or restrained with weights on top which were periodically removed (dynamic condition).

After 2 weeks, the resulting tissue engineered prosthesis was tested for:

1. Cell attachment using Alamar Blue (Vital stain) and DAPI (nuclear stain).
2. Collagen production using Sirius red staining.
3. Biomechanical properties (ultimate tensile strength, strain and Young's Modulus) using BOSE electroforce tensiometer.
4. Scaffold contraction using serial photographs.
5. Immunostaining for collagen I, III and elastin.

Results

Figure 1. (**a**) Metabolic activity,of OFs and ADSCs attached on Th PLA under free, static and dynamic conditions over two weeks culture, stained with AlamarBlue (n=9±SEM). (**b**)Production of total collagen by OFs, ADSCs and a mixture of both cells cultured on Th PLA under free, static and dynamic conditions. Sirius red staining after 14 days (n=9±SEM).

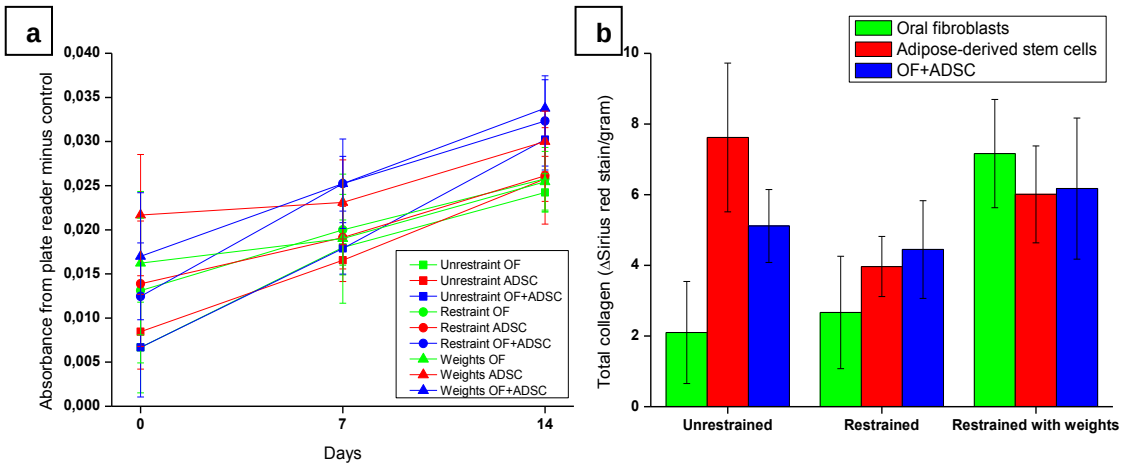


Figure 2. Biomechanical properties of Th PLA scaffolds cultured with OFs, ADSCs and a mixture of both under free, static and dynamic conditions for 14 days (n=9±SEM). Native tissue values are represented by 2 dashed lines (the range for native healthy paravaginal tissue) (3). (**a**) Young's modulus. (**b**) Ultimate tensile strength. (**c**) Strain at ultimate tensile strength.

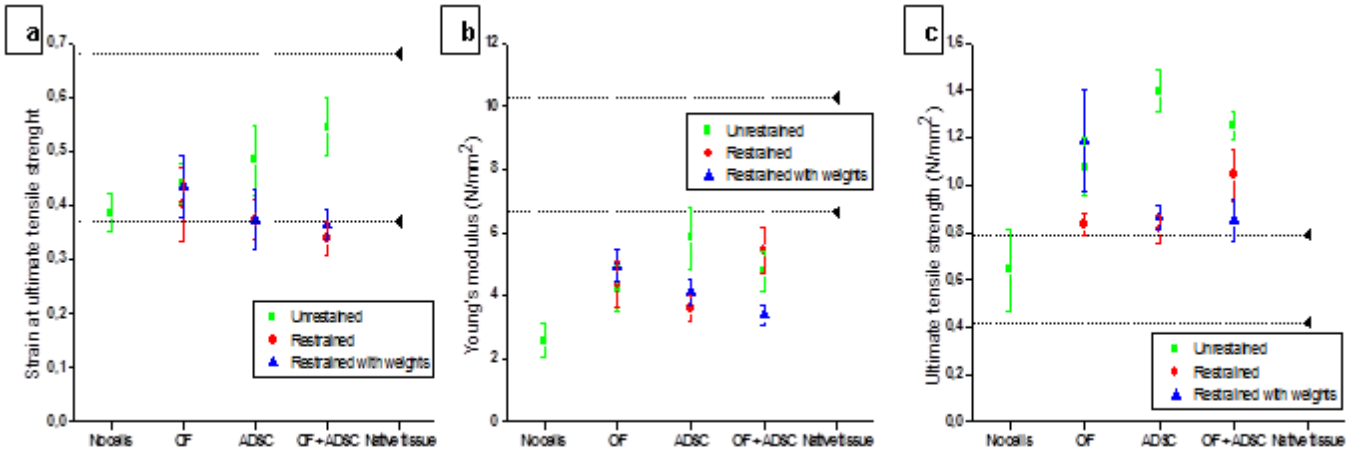
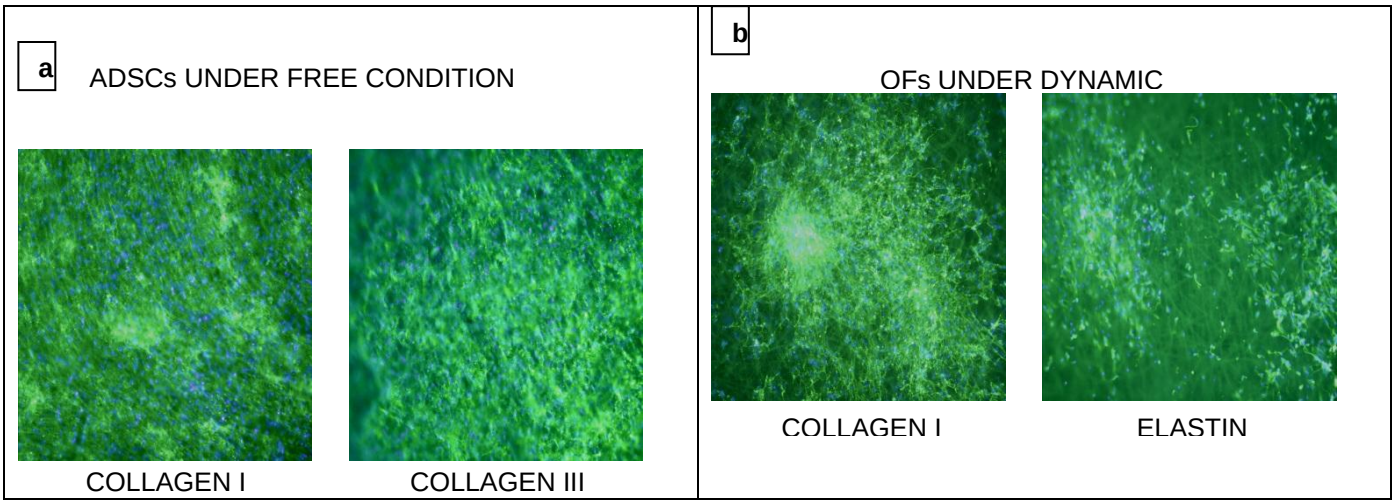


Figure 3. Presence and distribution of fibres of collagen I, III and elastin (green color) with cell nucleus (blue color) using immunostaining and DAPI respectively, after 14 days of cell culture in Th PLA scaffolds. (**a**) ADSCs under free condition. (**b**) OFs under dynamic condition.



Interpretation of results

Both cell types, OFs and ADSCs, showed good cell confluence (by DAPI images) and proliferation with good metabolic activity on PLA scaffolds. However, while ADSCs produced higher amount of total collagen under free condition, OFs are strongly stimulated to produce ECM under dynamic condition. The same is revealed from biomechanical properties where ADSCs achieved the strongest tissue engineered prosthesis with young's modulus closest to the native tissues.

Immunostaining showed very good ECM distribution for collagen I and III when ADSCs were cultured under free condition; and again, higher ECM components production for OFs under dynamic condition, but not so homogenously. On the other hand, OFs had greater elastin production, compared with ADSCs, under dynamic conditions when they were cultured alone and mixed with ADSCs.

Concluding message

In summary we seek to have tissue engineered prosthesis with good biomechanical properties at time of implantation. However, going beyond the current *in vitro* work we now need to consider issues of implantation. This will require looking at tissue integration, immune response, neovascularisation and the production of new ECM *in vivo* for lasting repair of SUI and POP.

References

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