INTRODUCTION:
Surgical repair of rotator cuff tendon tears has failure rates that are estimated to be as high as 75%. In late disease stages, this has been shown to be due to reduced cellularity and matrix organization [1]. The addition of biological factors such as stem cells and platelet rich plasma may improve the biological environment around the healing tendon [2]. We have investigated the use of an electrospun polydioxanone (PDO) scaffold patch to restrain the spread of injected biological factors, and retain them in the desired locality. The patch is biocompatible and biodegradable, and provides a confined space into which biological factors can be injected and retained. PDO has been shown to be biocompatible with tendon derived cells, and in an electrospun form, has been shown to have the required cell adhesion properties. This study characterizes the degradation of PDO microfibrous patches in vitro. We have used imaging techniques and high performance liquid chromatography to demonstrate the kinetics of PDO degradation as well as show the time scale of degradation in an in vitro representation of the physiological environment.

METHODS:
In-vitro weight loss Electrospun polydioxanone (Produced in collaboration with nanoforce) was used in this experiment along with controls of PDSII suture (Ethicon). The samples were sterilised in 70% ethanol. Test tubes were weighed using a Mettler AE 200 electronic analytical balance before having sterile samples added to them. Sterile PBS (Biowhitaker/Lonza) was then added in a ratio of 10mg/ml. The samples were incubated at 37°C and 5% CO₂. They were removed at intervals of 1 to 5 days, dried in an oven at 58°C, and then re-weighed. Samples were then re-sterilised before continued incubation. Weight loss of PDO samples was calculated as a percentage of the starting dry weight. The same experiment was repeated with PDO patch samples that were continuously incubated (without being weighed) for over 3 weeks before being re-weighed.

Imaging PDO samples were mounted on stubs, gold spattered and viewed in an Environmental Scanning Electron microscope (ESEM). Comparisons were made between fresh samples of PDO, and those degraded over a period of weeks in phosphate buffered solution (PBS). The dimensions of the imaged fibres were measured and compared in order to characterize how they degraded. Fluorescent microscopy (eclipse TE300, Nikon) was used to image patches into which seeded with tendon-derived cells. Cells were fixed in 10% formalin (Fisher Scientific), permeabilized and stained using DAPI (4',6-diamidino-2-phenylindole, Molecular Probes) and Rhodamine-Phalloidin (Invitrogen) These patches were incubated in medium and imaged after 1, 2, and 4 weeks to demonstrate the adhesion of the cells to the electrospun fibres as the PDO degraded.

High Performance Liquid Chromatography HPLC was used to confirm glyoxylic acid as the final breakdown product of PDO. pH PDO patches were incubated in PBS for a period of 8 weeks. At incremental time points, the pH of the solution was assessed. The Dynamic arm of the experiment involved replacing the PBS at each time point, in order to replicate the dynamic environment found in vivo. The Static arm maintained the same solution throughout the experiment.

RESULTS:
Our results indicated that the PDO patches lost the majority of their mass after 4 weeks of in vitro incubation (Figure 1). Similarly, the pH of the PDO/PBS solution fell most significantly after the 4th week (Figure 2). The ESEM images demonstrated the character of the PDO degradation, with degraded samples presenting short blocks of PDO in contrast to the long fibres of the fresh samples (Figure 3).

DISCUSSION:
In the study presented above, we characterized the time-scale of PDO degradation in a physiological solution using weight-loss and pH measurements. We demonstrated that tendon-derived cells survived on the electrospun scaffold even after 4 weeks of degradation. Moreover, the electrospun PDO structure maintained weight and pH well for 4 weeks which is the main window for physiological benefit from biological factors incorporated into the patch. After this time, it degrades to the final degradation product of glyoxylic acid.

SIGNIFICANCE:
This study improves our understanding of the degradation of a novel polydioxanone patch. It identified the end products and described the degradation rate and kinetics, as well as the morphology of the patch as it degrades. We expect this study to greatly assist in tailoring the electrospun patch to its specific function as a delivery and confinement device for tendon repair.

REFERENCES: