COMPARISON OF NOVEL CARBON-BASED SCAFFOLDS TO GRAFTJACKET IN TENDON REPAIR

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Introduction

A large part of the human body comprises of connective tissue (skin, ligaments, tendon, bone, fascia and cartilage. Advances in developing constructs that simultaneously fulfill the biologic needs for tissue healing and the mechanical strength needed for support have been slow. Natural collagen grafts and processed tissue grafts such as Graft Jacket, Tissuemend®, Restore Graft® and ZCR Patch Graft® do not meet requirements as materials for primary use where high mechanical strength is needed [1,2,3]. GraftJacket is only FDA approved to be used as augmentation membranes [1,3]. Carbon scaffolds are logical candidates as connective tissue substitutes. Recent applications of carbon scaffolds and implants include use in medical implants [4], repair sutures [5], and as vehicles for drug delivery [6]. Potential advantages of an engineered carbon scaffold may include the following: 1) tailored surface characteristics to meet *biological requirement* of a healing tissue 2) consistent *mechanical* properties to meet specific functional demands 3) eliminate the inherent compromise between increased mechanical stiffness and biology efficacy of connective tissue matrixes. The current study examines the physical and biological characteristics of carbon scaffolds as a potential primary scaffold that balances structure and property needs for connective tissue repair.

Experimental

Two GraftJacket® samples were provided by Wright Medical Technology. The carbon scaffolds were made from PAN carbon fibers. The first scaffold material (veil) was processed using milled fibers and bonded together, in a random fiber matrix, with a biocompatible polyester and then coated with a 5% solution of Polycaprolactone (PCL). The second scaffold material (fabric) was processed by a needling technique, bonded with polyester and then coated with a 5% solution of PCL. Samples were characterized using the MicroCT X-ray. MicroCT images of all the scaffolds are shown in Fig 1. The mechanical properties of samples were measured using MTS Mechanical Tester at a rate of 2.54 mm/min. Biological analysis was performed by seeding sample with approximately 50,000 fibroblast cells (CRL-153, ATCC) per sample and were grown in an incubated environment (37℃, 5% CO_2) for a period of 96 hours. The cells were cultured in F12K growth media (Fisher Scientific) with 10% FBS and 1% Penicillin/Streptomycin antibiotic. Cells were stained with a cell membrane (rhodamine phalloidin) and nuclear (DAPI) stain. Cell counts were accomplished with Metamorph Cell Imaging Software and confirmed with a WST-1 cell viability assay. Results from the porosity analysis, tension testing and biological analysis were expressed as means ± SD for each experimental group. For each Graft Jacket, six samples were tested while for each carbon-based scaffold, 10 samples were tested. Multiple comparison procedures were made by one-way analysis of variance (ANOVA). P-values equal to or less than 0.05 were considered significant.

Results and Discussion

GraftJacket samples were characterized and compared to carbon fiber scaffolds. As shown in Table 1, both GraftJacket groups display non uniform porosity distribution. While carbon sample porosity was more consistent. Tensile tests revealed that both fabrics exhibited higher stress at failure and the maximum load was significantly ($p < 0.001$) similar to the thicker GraftJacket sample group (Fig. 2, Fig. 3). Veil scaffolds exhibited lower stress and lower failure loads compared to both fabric and GraftJacket groups (Fig. 2, Fig. 3). Cellular growth studies revealed that there is a significant difference ($p < 0.05$) between GraftJacket samples and carbon scaffolds. However carbon fabric scaffolds still exhibited strong biocompatibility and growth (Fig. 4). Fibroblast growth on carbon scaffolds revealed a significant relationship ($p < 0.005$) between cell growth and porosity (Fig. 4). At 96 hours, both carbon groups of lower porosity exhibited greater cell growth.

There are many components of a tissue scaffold that can affect its behavior in vitro and in vivo; porosity, orientation, and biological activity. Many researchers have shown that the porosity of the scaffolds could affect cellular integration [7,8,9]. This study has illustrated that decreasing the porosity of the veil and fabric has a significant impact on the attachment and growth of fibroblast cells (Fig. 4). Moreover, varying materials porosity will have a direct impact on the mechanical behavior. This is evident from the direct correlation of the porosity to the tensile properties of both carbon scaffold groups (Fig. 2, Fig. 3). The ability of carbon fabric to exhibit similar load failure and greater stress than GraftJacket is very promising because one critical problem that arises in tissue scaffold design is compromising strength with decreasing size. The ability to increase strength but decrease size is a very challenging task however, this study illustrates that a compromise between decreasing thickness and increasing or maintaining strength is possible. The standard deviations of GraftJacket, represented in Table 1., also introduce challenges with applications where precise loads are needed. Not being able to predict when a material will fail could have catastrophic effects. Both carbon scaffold groups were engineered and have precise modes of failure and can be predicted and tailored to specific applications.

Carbon materials specifically, PAN carbon fibers have been shown to be biocompatible [10]. Our group has previously shown that cell growth is dependent on material orientation and that more oriented carbon materials attach and sustain greater cell growth [11]. The biological analysis

revealed that all carbon materials are biocompatible however carbon fabric materials exhibited greater cell growth than both veils (Fig. 4). Only at 96 hours does the GraftJacket material show significantly greater ($p < 0.001$) cell growth however both fabric materials still show strong signs of cell integration.

Table 1. Scaffold Properties

Material	Density (g/cm³)	Porosity (%)	Thickness (mm)
Graft Jacket	1.1-1.4	30 ± 20.2	0.46 ± 0.15
Graft Jacket	1.1-1.4	30 ± 20.2	2.0 ± 0.18
Carbon Veil 1	0.5	94 ± 1.2	0.3 ± 0.3
Carbon Veil 2	0.6	80 ± 3.7	0.32 ± 0.2
Carbon Fabric 1	0.8	55 ± 8.9	0.40 ± 0.2
Carbon Fabric 2	0.7	70 ± 6.2	0.45 ± 0.3

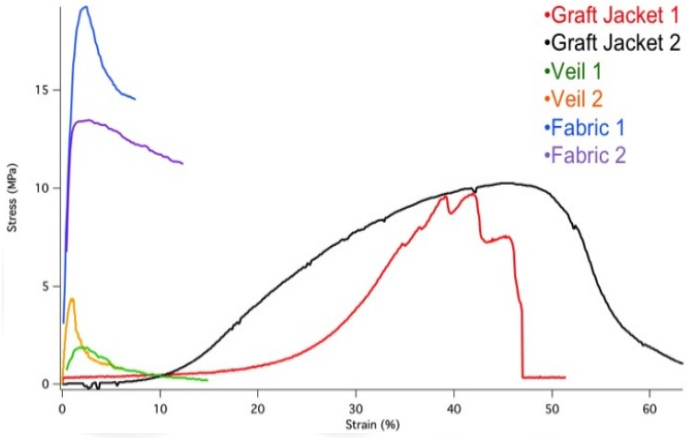

Fig. 2 Representative Tensile Test Data for Scaffold Groups.

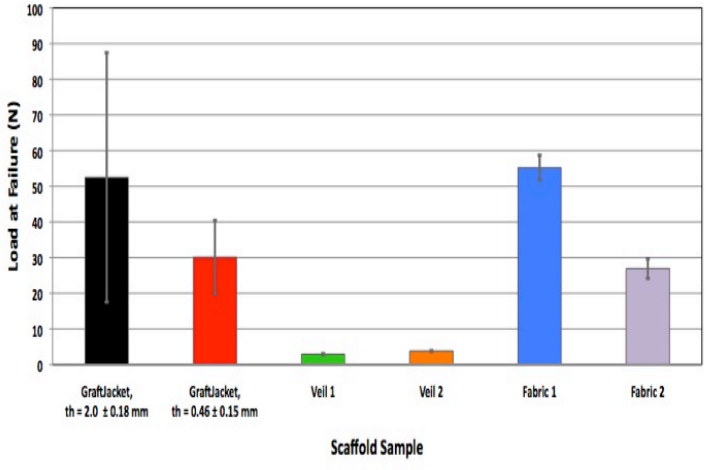

Fig. 3 Load Failure for Scaffold Groups.

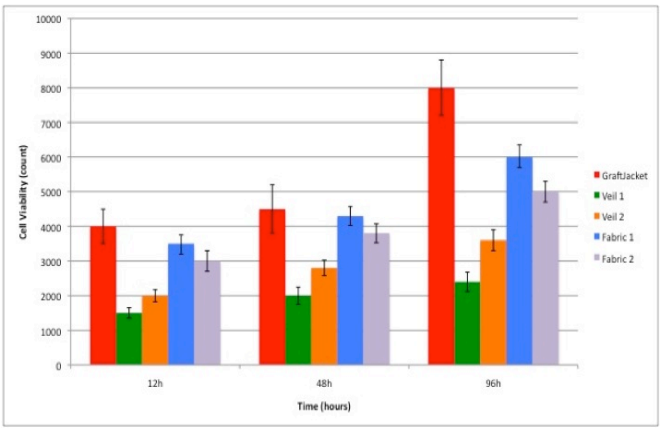

Fig. 4 Fibroblast growth on Scaffold Samples.

Conclusions

Tissue scaffolds need to have minimal antigenicity, sutable mechanical properties, and promote revascularization and conversion to host tissue. Manipulating the structure and material type will dramatically change how these materials behave mechanically and biologically. Two carbon fiber derived scaffolds, varying in porosity, were successfully processed and compared to two GraftJacket groups of varying thickness. GraftJacket is a FDA approved tissue membrane that is not approved as a primary scaffold in load bearing applications. Porosity analysis results reveal that GraftJacket has a large standard deviation whereas both fabric and veil could be tailored and controlled. The maximum tensile stress of fabric is significantly greater than GraftJacket and the load failures are significantly simliar. Biological analysis revealed that all carbon materials are biocompatible and fabric materials show strong cell growth after 96 hours. Carbon materials, with controlled structure and porosity could be modified and tailored to balance strength and biological activity.

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