Introduction: Porous three-dimensional scaffolds play an important role in tissue engineering of malfunctioning or lost organs. Naturally derived biodegradable collagen and synthetic biodegradable poly(alpha ester)s like poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymers of poly(DL-lactic-co-glycolic acid) (PLGA) have been widely used to construct temporary scaffolds for tissue engineering. PLA, PGA and PLGA are biocompatible and approved by the Food and Drug Administration (FDA) for certain human clinical applications. They are easily processed into desired shapes with good mechanical strength. Their degradation periods can also be manipulated by controlling the crystallinity, molecular weight, and copolymer ratio of lactide to glycolide acid. Despite these advantages, PGA, PLA and PLGA-derived scaffolds lack cell-recognition signals, and their hydrophobic property hinders smooth cell seeding. On the other hand, naturally derived collagen has the potential advantage of specific cell interactions, but offers limited versatility in designing a scaffold with specific physical properties. PLGA-derived scaffolds lack cell-recognition signals, and their hydrophobic property hinders smooth cell seeding. On the other hand, naturally derived collagen has the potential advantage of specific cell interactions, but offers limited versatility in designing a scaffold with specific physical properties such as mechanical strength. In this study, a hybrid sponge of PLGA and collagen was fabricated by forming microsponges of collagen in the pores of a PLGA sponge and used for three-dimensional in vitro culture of chondrocytes.

Materials and Methods: The PLGA sponge was first prepared by a particulate-leaching technique by adding NaCl particulates (9 g), ranging in diameter from 355 to 425 um, to a PLGA solution in chloroform at a concentration of 20 (w/v)% and leaching them out of the dried PLGA/NaCl composite. Then the PLGA sponge was immersed in type I collagen acidic solutions (pH 3.2) under a vacuum so that the sponge pores filled with collagen solution. The collagen solution-containing PLGA sponge was then frozen at -80 °C for 12 hours, and lyophilized under a vacuum of 0.2 Torr for an additional 24 hours to allow the formation of collagen microsponges in the sponge pores. The collagen microsponges were further cross-linked by treatment with glutaraldehyde. The dried PLGA-collagen hybrid sponge was fabricated. The structures of both the PLGA and the PLGA-collagen hybrid sponge were observed by scanning electron microscopy (SEM) and SEM-electron probe microanalysis (SEM-EPMA). Their wettabilities with water were assessed by measuring the contact angles of water on PLGA film and collagen-coated PLGA film. Dried and 20 nM HEPES buffer-soaked PLGA, collagen, and PLGA-collagen hybrid sponges were used for static tensile and compression mechanical tests. Chondrocytes were isolated from articular cartilage of a calf knee joint by collagenase digestion and seeded into the PLGA-collagen hybrid sponge. The cell seeded hybrid sponges were placed in culture flasks with 40 mL Dulbecco’s modified Eagle’s medium and cultured at 37°C/5%CO2. The medium was changed every three days. After chondrocytes were cultured for varying time intervals, wells were fixed in 10% paraformaldehyde and observed by SEM. Their 10-μm histologic cross sections were stained with hematoxylin and eosin, or immunostained with anti-type II collagen antibody.

Results: SEM observation demonstrated that the PLGA sponge had a uniformly distributed and interconnected pore structure. The pore size was equal to the size of the NaCl particulates used. The porosities were approximately equal to the initial sodium chloride weight fractions. Microsponges of collagen with interconnected pore structures were formed in the pores of the PLGA sponge (Fig.1). The porosity decreased slightly after the introduction of collagen microsponge. The hybrid structure of the PLGA-collagen hybrid sponge was further confirmed by detecting elemental nitrogen, which exists in collagen but not in PLGA copolymer, with SEM-EPMA. Nitrogen element was detected in the microsponges of collagen and the pore surfaces of PLGA pores, but not in the cross-sections of PLGA regions. This indicates that microsponges of collagen were formed in the pores of PLGA sponge and that the pore surfaces were also coated with collagen. The formation of collagen microsponges in the pores of PLGA sponge relied on the concentration of collagen solution. The effective concentration of collagen solution was in the range from 0.1 to 1.5 (w/v)%.

Discussion: Because both natural and synthetic polymers have their respective advantages, much attention has been focused on the synthesis of composites of natural and synthetic polymers to combine the advantageous properties of both constituents. This study demonstrated that a biodegradable hybrid sponge of PLGA and collagen was fabricated by forming microsponges of collagen in the pores of PLGA sponge. The hybrid sponge was reinforced by collagen microsponges and its wettability with water was improved, which facilitated cell seeding in three dimensions. Chondrocytes adhered well and spread on the surfaces of the microsponges of collagen in the hybrid sponge. The distribution of cells was uniformly throughout the hybrid sponge. Use of the PLGA sponge as a skeleton facilitated formation of the hybrid sponge into the desired shapes with high mechanical strength, while collagen microsponges contributed good biocompatibility and hydrophilicity.

References: