In Vitro Comparison of Biological and Synthetic Materials for Skeletal Chest Wall Reconstruction

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Background. **Various biological and synthetic materials have been proposed for use in skeletal chest wall reconstruction (SCWR). Because of the lack of studies allowing a direct comparison of SCWR materials, their clinical use often depends on the surgeon's preference and experience. The aim of this study was to analyze 6 synthetic and 3 biological materials frequently used in SCWR with respect to their cytotoxicity, bacterial adhesion, surface characteristics, and mechanical properties to facilitate data-driven decisions.**

Methods. **The effect of the SCWR materials and their extracts on the metabolism of human skeletal muscle cells (SkMCs), dermal fibroblasts, adipose cells, and osteoblasts was analyzed in vitro. Bacterial adhesion was quantified by incubating samples in bacterial suspensions (*Staphylococcus epidermidis*, *S aureus*, and *Escherichia coli*), followed by counting colony-forming units and performing scanning electron microscopy. Moreover, the mechanical properties of the materials were analyzed under uniaxial tensile loading to failure.**

Results. **The metabolism of all cell types seeded on the SCWR materials was reduced compared with untreated cells. With the exception of Vypro (Ethicon, Somerville, NJ), whose extracts significantly reduced fibroblast viability, no cytotoxic leachable substances were detected. Biological materials were less cytotoxic compared with synthetic ones, but they demonstrated increased bacterial adhesion. Synthetic materials demonstrated higher elongation to failure than did biological materials.**

Conclusions. **Biological and synthetic SCWR materials showed significant differences in their cytotoxicity, bacterial adhesion, and biomechanical properties, suggesting that they may be used for different indications in SCWR. Further comparable in vivo studies are needed to analyze their performance in different indications of clinical application.**

(Ann Thorac Surg 2015;■:■–■)

Depending on the size, localization, and different indications of chest wall defects, tissue adaption and skeletal chest wall reconstruction (SCWR) is needed [1, 2]. The ideal material for SCWR must fulfill numerous criteria—among other things, biocompatibility and mechanical stability [2–4]. Although a variety of different materials have been used, there are currently no guidelines for the selection of the appropriate material for different indications. The literature indicates only that along with other substances, artificial materials promote foreign body reactions, interfering with wound healing and material incorporation and resulting in an increased risk of mortality and morbidity [5–7].

Additionally, manufacturers disclose limited information about the tests performed on approved SCWR materials, making comparison impossible. Although various case studies described frequently used materials [8–17], studies comparing such materials under standardized conditions have not been reported or only partially implemented [18–20]. Thus ultimately the choice of the SCWR material usually depends on the personal preference and experience of the surgeon [2].

Therefore, the present study aimed to analyze essential properties of 9 commercially available SCWR materials under standardized in vitro conditions. Hence, the cytotoxicity of the selected materials against cell types involved in material incorporation—namely, primary normal human dermal fibroblasts (NHDFs), human osteoblasts (HOBs), skeletal muscle cells (SkMCs), and human white preadipocytes (HWPs)—was tested. Because of their responsibility for wound healing disorders, adhesion of *Staphylococcus epidermidis, S aureus,* and *Escherichia coli* to the respective material surface was investigated. Finally the mechanical properties of these materials were analyzed under uniaxial tensile loading to failure.

Accepted for publication Sept 19, 2014.

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Published by Elsevier

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2014.09.040

Abbrevations and Acronyms

CFU = colony-forming unit
HOB = human osteoblasts
HWA = human white adipocyte
HWP = human white preadipocyte
MSD = minimum significance difference
MTS = CellTiter 96 AQueous One Solution Cell Proliferation Assay
MV = mean viability
NHDF = normal human dermal fibroblasts
SCWR = skeletal chest wall reconstruction
SEM = scanning electron microscopy
SkMC = skeletal muscle cell
TSB = Tryptone soya broth

Material and Methods

Materials

The biological materials Peri-Guard (Synovis Surgical Innovations, St. Paul, MN), VERITAS Collagen Matrix (Baxter, Deerfield, IL), and Permacol (Covidien, Mansfield MA) and the synthetic materials Vypro (Ethicon, Inc, Somerville, NJ), Vicryl (Ethicon Inc), Gore-Tex (W.L. Gore & Associates, Flagstaff, AZ) Premilene (Braun, Tutlingen, Germany), Parietex Composite (Covidien), and Parietene (Covidien) were analyzed.

Cytotoxicity

In vitro cytotoxicity tests were conducted according to International Organization for Standardization (ISO) 10993-5, which is a requirement for biological safety evaluation of all medical devices before market access in the European Union (DIN EN ISO 10993-12: Biological evaluation of medical devices–Part 12: Sample preparation and reference materials [ISO 10993-12:2009]; DIN EN ISO 10993-5: Biological evaluation of medical devices–Part 5: Test for in vitro cytotoxicity [ISO 10993-5:2009]).

Cell Culture

Normal human dermal fibroblasts (NHDFs) (PromoCell, Heidelberg, Germany), human osteoblasts (HOBs) (PromoCell), skeletal muscle cells (SkMCs) (PromoCell), and human white preadipocytes (HWPs) (PromoCell) were standardized and cultured at 37°C, 5% CO_2, and 90% relative humidity in their respective culture media (PromoCell) according to the manufacturer's instructions. Before the experiments, HWPs were differentiated from preadipocytes by culturing them for 3 days in Preadipocyte Differentiation Medium (PromoCell). Their differentiation status was confirmed by monitoring lipid droplets. Thereafter, mature human white adipocytes (HWAs) were cultured in Adipocyte Nutrition Medium (PromoCell).

Contact Cytotoxicity of SCWR Materials

Cells were seeded onto aseptic disks (10-mm diameter) of each material at a density of 1.6×10^4/mL (NHDFs), 2×10^4/mL (SkMCs and HWAs), and 3×10^4/mL (HOBs). Glass coverslips (10-mm diameter) were used as negative controls (100% viability) and RM-A (cytotoxic control) (Hatano Research Institute, Kanagawa, Japan) as positive controls. After 3 days of standard incubation, cell viability was measured using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) (Promega Corp, Madison, WI). Eight test items were analyzed in parallel. Culture medium was used as a background control and subtracted from all values before calculating the means and 95% confidence intervals.

Extract Preparation of SCWR Materials and Their Cytotoxicity

To test the release of soluble toxic substances by the materials, liquid extracts were prepared in the respective culture media using a weight-to–extraction volume ratio of 0.2 g/mL. As control samples, RM-A and RM-C (noncytotoxic negative control) (Hatano Research Institute, Kanagawa, Japan) were extracted with a surface-to–extraction volume ratio of 3 cm^2/mL. Culture media were incubated in parallel as negative and background controls. All test, control, and background samples were extracted for 72 hours at 37°C. The respective cells were seeded at a density described earlier in 96-well plates and incubated in a standard manner. After 24 hours, the respective culture media were replaced by the extracts. After 3 days of incubation, cell viability was measured using MTS. The experiments were performed in quadruplicate. Culture medium was used as a background control and subtracted from all values before calculating means and 95% confidence intervals. Cells incubated with only growth media were used as negative controls (100% viability).

Bacterial Adhesion

BACTERIAL STRAINS AND IN VITRO INCUBATION. Isolates of *Staphylococcus epidermidis* (ATCC 14990) and *S aureus* (ATCC 12600), as typical skin germs, and gram-negative *Escherichia coli* (ATCC 25922) were cultured on blood agar plates at 37°C for 24 hours before measurement of the bacterial concentration by optical density (BioPhotometer, Eppendorf International, Hamburg, Germany). Subsequently, several colonies per strain were extracted and emulsified in Tryptone soya broth (TSB) (Thermo Scientific, Hampshire, UK) to obtain an optical density of 0.5 (measured at 600 nm). Aliquots (1.5 mL) of each bacterial suspension were placed into 24-well plates. Samples ($n = 4$) of each material and bacterial strain were transferred to these plates and incubated at 37°C for 24 hours.

DIRECT COUNTING OF COLONY-FORMING UNITS. After incubation, the samples were removed and gently washed 3 times in phosphate-buffered saline and transferred into sterile universal containers containing 5 mL of phosphate-buffered saline in which adherent bacteria were released by sonication at low power for 20 minutes. This procedure has been found to remove all adherent bacteria without affecting their viability (data not shown). Once bacteria were removed, the bacterial suspension