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# Effective Cell-Seeding Technique Using Magnetite Nanoparticles and Magnetic Force onto Decellularized Blood Vessels for Vascular Tissue Engineering

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**Increasing attention has been given to vascular tissue engineering in recent years. Although cell seeding onto tubular scaffolds is the first step for constructing three-dimensional vascular grafts, the tubular geometry of the grafts hinders the efficient delivery of cells onto the scaffold. To overcome these limitations, we present here a novel cell-seeding technique using magnetic force and magnetite nanoparticles, termed Mag-seeding. NIH/3T3 fibroblasts (3T3s) were labeled magnetically using our original magnetite cationic liposomes (MCLs), which have a positive surface charge, to improve adsorption onto cell surface. In this study, porcine decellularized common carotid artery (dCCA) was used as one of the most promising scaffolds, because dCCA consists of a mixture of structural and functional proteins that constitute the extracellular matrix. When a cylindrical magnet was inserted into the lumen of dCCA and the dCCA was immersed into a suspension of magnetically labeled 3T3s, almost all the 3T3s attached onto the dCCA, whereas a low cell-seeding efficiency was achieved without using a magnet. When the magnetite uptake rate per cell increased, cell-seeding efficiency by Mag-seeding was enhanced. Furthermore, to construct a vascular graft for humans, the porcine dCCA, which was reseeded with two human cells (smooth muscle cells and dermal fibroblasts), was successfully constructed by Mag-seeding. These results indicate that Mag-seeding can be used for vascular tissue engineering.**

[**Key words:** vascular tissue engineering, magnet, magnetite nanoparticles, decellularized blood vessel, cell seeding]

Tissue engineering has been given much attention and investigated for the regeneration of many kinds of tissue and organ (1). In vascular tissue engineering, pioneered by Weinberg and Bell (2), many researchers have explored the use of arterial tissue cells combined with scaffolds to construct tissue-engineered blood vessels (TEBVs). One typical approach for constructing TEBVs is to seed three types of cells, namely, endothelial cells (ECs), smooth muscle cells (SMCs) and fibroblasts, onto tubular-shaped synthetic or natural scaffolds (3). Several research studies suggest that ECs seeded onto the lumen surface of a tubular scaffold prevent thrombus formation following implantation, and that SMCs and fibroblasts seeded onto the scaffold improve the host-graft interaction and offer advantages into mechanical strength (3–7).

Cell seeding onto tubular scaffolds is the first and an important step for vascular tissue engineering; however the tubular geometry of the grafts hampers the efficient delivery of cells onto the scaffold. To overcome these limitations, various techniques of cell seeding onto tubular scaffolds have been investigated. For recent examples, McFetridge *et al*. (8) compared three independent SMC-seeding techniques, namely, rotated bioreactor, static-seeding and cell sheet wrapping techniques; Soletti *et al*. (9) developed a seeding device for a tubular structure that uses the synergistic effects of vacuum, centrifugal force and flow; Kitagawa *et al*. (10) developed a radial-flow perfusion bioreactor for vascular reconstruction. Although these seeding techniques seem to have the potential to advance vascular tissue engineering, novel easy, simple, and highly efficient cell-seeding techniques for tubular scaffolds are required.

Some synthetic materials, such as polyethylene terephthalate (PET), expanded polytetrafluoroethylene (ePTFE),

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and so on, have been successfully used in clinical applications with a blood vessel diameter range >5–6 mm. However, these materials are not applicable to small-diameter blood vessels (<5 mm) because their mechanical properties are different from those of natural blood vessels (4). Several studies have indicated that the differences in mechanical properties have detrimental effects on biomedical adaptation (11, 12). Therefore, the scaffolds for small-diameter TEBVs must be biocompatible (*i.e.*, nonimmunogenic, resistant to infection, and facilitate cell attachment) and possess mechanical properties, compliance and stiffness that are similar to those of natural blood vessels. Although many synthetic or biological scaffolds have been examined for creating more reliable alternative small-diameter TEBVs, optimal scaffolds have not been developed yet (3–6, 13, 14). Recently, the utility of decellularized tissues has been elucidated and investigated for various tissue engineering applications (15, 16). In vascular tissue engineering, because decellularized blood vessels, the common carotid artery (CCA), the external jugular vein and so on, remain natural structural and functional extracellular matrixes (ECMs), decellularized blood vessels seem to have great potential as scaffolds (8, 17–19). In our previous studies, we evaluated the compliance, which is the ability for passive expansion and contraction with changes in pressure constitutes, of five materials, namely, a native blood vessel (CCA), two synthetic materials (*i.e.*, E-PLA (an elastin gel combined with polylactic acid) and ePTFE), and two decellularized matrixes (*i.e.*, dCCA and D-Ureter (a decellularized ureter)), using intravascular ultrasound and it was shown that E-PLA and ePTFE did not exhibit a compliant response ($1.9 \pm 3.3\%$/100 mm Hg for E-PLA and $0.83 \pm 0.21\%$/100 mm Hg for ePTFE), whereas dCCA ($17.8 \pm 3.2\%$/100 mm Hg) and D-Ureter ($6.0 \pm 2.5\%$/100 mm Hg) exhibited coequal compliance with CCA ($12.2 \pm 2.0\%$/100 mm Hg) (20). Therefore, dCCA has a great potential as a scaffold of TEBVs. Because some reports showed that decellularized blood vessels reseeded with vascular cells remained patent *in vivo* for a longer period than those without reseeding (17, 18), the development of a cell-seeding method onto dCCA is needed to construct promising TEBVs.

Our research group has proposed a novel tissue engineering methodology using magnetic force and magnetite nanoparticles, and is termed as Mag-TE (21). Mag-TE can be divided into two processes: (i) labeling cells magnetically using magnetite cationic liposomes (MCLs) and (ii) manipulating magnetically labeled cells directly using a magnetic force. MCLs are cationic liposomes containing 10-nm magnetite nanoparticles and they improve the accumulation of magnetite nanoparticles in target cells via electrostatic interaction with a negatively charged cell membrane. Magnetically labeled cells are accumulated by the magnetic force onto the targeted place to successfully construct several kinds of multilayered sheetlike construct, such as keratinocyte (22), retinal epithelial cell (23), cardiomyocyte (24), dermal fibroblast (25), and mesenchymal stem cell (MSC) sheets (26). On the basis of the concept of Mag-TE, we previously developed an effective cell-seeding technique named Mag-seeding (27). Briefly, a magnet is placed on the reverse side of the culture dish of which a three-dimensional cylindrical porous scaffold is placed on the center; because magnetically labeled cells using MCLs are attracted by the magnet, magnetically labeled cells poured onto the top surface of the scaffold could be seeded successfully at a high density (27).

In this study, we applied Mag-seeding to a tubular scaffold, namely, a porcine decellularized common carotid artery (dCCA). Magnetically labeled NIH/3T3 fibroblasts (3T3s) as a model cell were used to evaluate the efficiency of Mag-seeding onto dCCA. We investigated whether the magnetite uptake rate by cells is correlated with cell-seeding efficiency onto dCCA. Moreover, magnetically labeled human cells (smooth muscle cells and dermal fibroblasts) were seeded onto the porcine dCCA by Mag-seeding for the construction of a recellularized vascular graft for humans.

## MATERIALS AND METHODS

**Cells and culture** NIH/3T3 fibroblasts (3T3s) were obtained from the American Tissue Culture Collection and were cultured in Dulbecco's Modified Eagle's Medium with a high glucose concentration (DMEM; Invitrogen, San Diego, CA, USA) containing 10% fetal bovine serum, 0.1 mg/ml streptomycin sulfate, and 100 U/ml potassium penicillin G (Invitrogen). Human normal aortic SMCs (Cambrex Bio Science Walkersville, Walkersville, MD, USA) were cultured in a smooth muscle cell growth medium (SmGM-2; Cambrex Bio Science Walkersville). Human normal dermal fibroblasts (NHDFs; Kurabo, Osaka) were cultured in Medium 106S (Kurabo). The cells were cultured at 37°C in a humidified atmosphere of 5% $CO_2$ and 95% air. The culture medium was changed every other day.

**Preparation and decellularization of porcine CCA** Porcine CCA was harvested from pigs (mean body weight, 11 kg) when they were euthanized for other experiments. The CCAs were decellularized with deoxycolic acid in accordance with a previously reported method with some modifications (28). Briefly, CCAs were placed in a 50-ml test tube and treated with 30 ml of phosphate-buffered saline (PBS) solution containing 0.1% sodium azide (Wako, Osaka). The tube was agitated at a frequency of 140/min for 12 h. The samples were transferred to a new test tube and treated with 30 ml of a PBS solution containing 5.8% NaCl (Wako), 0.02% DNase (Roche, Basel, Switzerland), and 0.01% RNase (Roche) for 12 h. Then, the samples were treated twice with 30 ml of a PBS solution containing 4% deoxycolic acid (Sigma-Aldrich, St. Louis, MO, USA) for 12 h. Subsequently, the samples were treated with 30 ml of a PBS solution with a 1% antibiotic antimycotic reagent (Invitrogen) and washed using a shaker for 3 d, changing the PBS solution every 12 h. In this study, the decellularized CCA (dCCA) was cut into 20-mm-long specimens before the experiments. All animal experiments were performed according to the Guidelines for Animal Experimentation of the Nagoya University School of Medicine.

**Preparation of MCLs** MCLs were prepared as described previously (29). Briefly, colloidal magnetite ($Fe_3O_4$; average particle size, 10 nm; Toda Kogyo, Hiroshima) was added to a lipid mixture consisting of *N*-(α-trimethylammonioacetyl)-didodecyl-D-glutamate chloride (TMAG, a cationic lipid, Sogo pharmaceutical, Tokyo), dilauroylphosphatidyl-choline (DLPC, Sigma-Aldrich), and dioleoylphosphatidyl-ethanolamine (DOPE, Avanti Polar Lipids, Alabaster, AL, USA) in a 1:2:2 molar ratio. Magnetite was used as the core of the MCLs. The average MCL particle size that was measured using a dynamic light scattering spectrophotometer (FRAR 1000; Otsuka Electronics, Osaka) was 150 nm.

**Magnet** A cylindrical magnet whose magnetic poles are on its curved surface was used (diameter, 3 mm; length, 10 mm; mag-