Species-Specific Collagen Deposition in Tissue-engineered Bone and Cartilage: A Xenograft Model of the Human Digit
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Significance: The quality of the heteropolymetric collagen framework is crucial if tissue-engineered or regenerated bone and cartilage is to withstand mechanical stress.

INTRODUCTION

Histochemical analyses have shown that tissue-engineered cartilage and bone can develop faithfully if biodegradable scaffolds seeded with chondrocytes or covered with periosteum are implanted subcutaneously in athymic (nude) mice (1, 2). Genes specific for transplanted species and tissues such as bovine or human are expressed (3) but species specificity of the corresponding proteins, for example, type I or type II collagen, deposited in extracellular matrices is still not clear. One reason for this uncertainty is the inability of antibodies to distinguish between homologous collagen chains in different species.

We have focused recent research efforts to address this question and whether a heteropolymeric collagen network is assembled in tissue-engineered cartilage. Using micro-methods in protein mass spectrometry we studied bovine chondrocytes and human osteoblasts cells implanted on biodegradable scaffolds deposit bovine-specific collagen in newly formed cartilage and bone when implanted in athymic (nude) mice. Additionally, using a newly developed method to monitor the quality of synthesized matrix by chondrocytes in culture, we show that in this engineered cartilage, collagen types II, IX and XI assemble into the co-polymorphic cross-linked network that typifies cartilage matrix in vivo.

METHODS

Preparation and implantation of tissue-engineered phalanges. Tissue-engineered constructs modeling human phalanges were prepared as described previously (1, 3). Briefly, articular chondrocytes dissociated from shoulders and forelimbs of newborn calves were seeded onto polyglycolic acid (PGA) polymer mesh sheets 1 x 1 x 0.2 cm in dimensions. Two sheets were sutured to both ends of a polyethylene tube (poly(L-lactic acid) (PLLA) scaffold) shaped into the form of a human middle phalanx. The midshaft was wrapped over half its length with a strip of normal bovine periosteum with its cambium layer in contact with the scaffold. The remaining half midshaft was left uncovered. Constructs were implanted into the dorsal subcutaneous space of 4-6 week old nude mice. Implants were removed after 20 weeks and the extracellular matrices formed in the tissue-engineered cartilage and bone were examined biochemically.

Collagen extraction. Collagen laid down by the bovine chondrocytes in the engineered cartilage was depolymerized and extracted by peptidase in acetic acid. The collagen deposited in construct midshafts was extracted by cyanogen bromide (CNBr). The various collagen chains and chain fragments were resolved by SDS-PAGE.

Mass spectrometry. In-gel trypsin digests of α1(II) and α1(XI) collagen chains from the tissue-engineered cartilage were analyzed to confirm bovine-specific type II and type XI collagen protein. In-gel trypsin digests of type I collagen CNBr-peptides from midshafts were analyzed to determine bovine-specificity.

Western blotting. To fingerprint cartilage-typic collagen heteropolymer formation, the collagen chains were transferred to PVDF and probed with monoclonal antibody (mAb) 10F2 which recognizes a cleavage site (neo-epitope) in a sequence in the C-telopeptide cross-linking domain of type II collagen (4) and mAb 2B4 which recognizes the cleaved C-terminal peptide of α1(XIX) collagen cross-linked to α1(II). Collagen types II and XI collagen from bovine cartilage were run as a standards.

RESULTS

Fig. 1A illustrates the gross appearance of glossy, firm, cartilaginous tissue at the ends of one specimen of an engineered middle phalanx model retrieved 20 weeks after implantation in a nude mouse. Histological analysis of the neo-cartilage showed intense Safranin-O staining. Rudimentary growth plate development occurred adjacent to midshaft periosteum. The midshaft showed strong alizarin red and von Kossa staining in periosteum-covered regions (1). Fig. 1B, an X-ray of the identical construct, shows mineralization present only within the region of the midshaft covered with periosteum.

CNBr digestion of midshaft collagen clearly showed a peptide pattern typical of type I collagen in both the periosteum-covered and uncovered regions. Most importantly, in-gel trypsin digests of select CNBr peptides followed by mass spectrometry identified peptides from the α1(I) and α2(I) collagen chains of bovine origin from the periosteum uncovered midshaft region as well as from within the periosteum-covered and mineralized region. As shown in Fig. 2, the amino acid residues are uniquely bovine. We conclude that cells from bovine periosteum had penetrated the scaffold and migrated into the uncovered region of the scaffold and deposited type I collagen there.

SDS-PAGE followed by Coomassie blue staining confirmed that type II collagen was the major collagen solubilized by peptidase from the construct neo-cartilage. Mass spectrometry of trypsin-treated collagen chains revealed peptides from α1(II) and α1(XI) collagen chains of bovine origin. The seeded chondrocytes remain differentiated and secreted an extensive extracellular matrix containing type II collagen. The total collagen content of tissue-engineered cartilage was 47% dry wt and approached bovine articular cartilage control values (52%).

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<th>Sequence by Mass Spectrometry</th>
<th>Sequence ID</th>
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<tr>
<td>GDNQAGAGPFGPGPFGPPGAGAR</td>
<td>α1(I)</td>
</tr>
<tr>
<td>GQPAIGAGPAGAGANGR</td>
<td>α2(I)</td>
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<tr>
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<td>α1(XI)</td>
</tr>
<tr>
<td>TQPPGGGVPQPPGTTGPIGER</td>
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Figure 2. Mass spectrometry of trypsin-digested CNBr peptides or intact collagen chains revealed sequences specific for bovine collagens. An example of one only peptide per collagen chain is shown here. Amino acids highlighted and underlined are unique for the bovine sequence.

In Western blots, mAb 10F2 reacted with cartilage α1(I) chains, a result indicating a cross-linked type II collagen network had formed in constructs by 20 weeks. The α1(XI) chain was also immunoreactive, showing that type XI collagen was co-polymerized and cross-linked to C-telopeptides of type II collagen. Peptidase extracts of normal bovine cartilage revealed a similar pattern. The mAb 2B4 reacts with the peptidase α1(II) chain meaning that α1(IX) chain was cross-linked to it in the constructs. Such cross-linking sites have been reported for normal articular cartilage (4). These data demonstrate that a type II/XI/XIX collagen heteropolymeric network as present in normal cartilage had developed. The hydroxylysyl-pyrindoline cross-link content (1.4 moles/mole of collagen) of the tissue-engineered cartilage was very close to control bovine articular cartilage (1.6 moles/mole of collagen).

DISCUSSION

The results reveal that bovine-specific collagen molecules are deposited in the matrix of bone and cartilage in the engineered constructs. Assembly of collagen II/XI/XIX heteropolymers in the neo-cartilage indicates bovine chondrocytes seeded in scaffolds and implanted in nude mice remain differentiated through 20 weeks. Bovine type I collagen was detected in midshafts, a result demonstrating viable cells originally from the covering periosteum continued to populate the scaffolds. Periosteum also clearly mediates mineral deposition in construct midshafts as only periosteum-covered regions showed mineral opacity in radiographs. The findings from this study support the use of human cells for future tissue engineering applications of phalanx models.

REFERENCES

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